

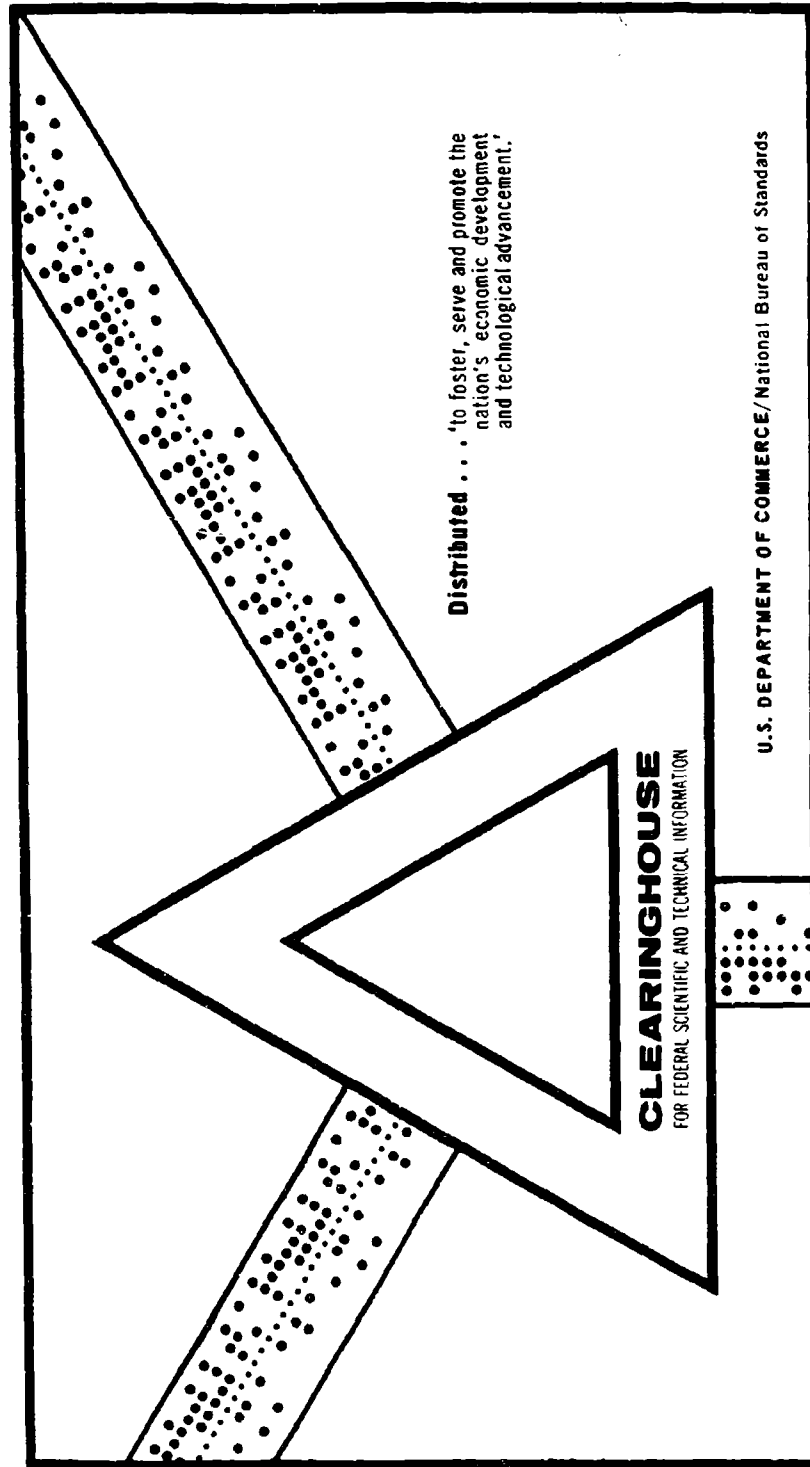
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# HAZARDS OF SMOKE AND TOXIC GASES PRODUCED IN URBAN FIRES

Andrew J. Pryor, et al

Southwest Research Institute  
San Antonio, Texas

September 1969



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by

A. J. Pryor  
D. E. Johnson  
N. N. Jackson

## FINAL REPORT

OCD Contract No. DAHC20-70-C-0212

OCD Work Unit 2537 B

SwRI Project No. 03-2402

Prepared for

Department of the Army  
Office of the Secretary of the Army  
Office of Civil Defense

September 1969

RESEARCH WAS CONDUCTED ACCORDING TO THE PRINCIPLES ENUNCIATED IN THE "GUIDE FOR LABORATORY ANIMAL FACILITIES AND CARE," PREPARED BY THE NATIONAL ACADEMY OF SCIENCES-NATIONAL RESEARCH COUNCIL.

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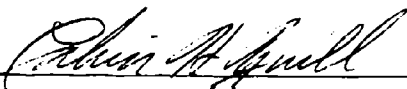
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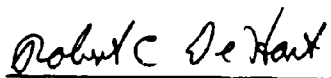
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## ABSTRACT

In previous studies, the significance of synergistic action brought about by animal exposures to combinations of elements found within the combustion products in a mass fire environment was identified. An experimental program was undertaken to further define the life hazard in a mass fire environment resulting from exposures to these combustion products. Studies included exposures to combinations including the variables of carbon monoxide, temperature, oxygen, carbon dioxide, sulfur dioxide, nitrogen dioxide, hydrogen cyanide, the presence of smoke (particulate matter), and all of the trace constituents to be found. The data from these tests and the results of the histopathological studies were reviewed in an effort to define the significance of human exposure to combustion products as found within the mass fire environment.

## SUMMARY

The hazard to life from combustion products experienced in the fire environment that may be brought about in time of conflict has been identified as a problem of serious proportion by civil defense interests. This program consisted of a series of animal exposure experiments designed to evaluate the significance of exposure to combinations of the variables found in this fire environment. An effort has been made to interpret these findings in terms of effects on humans.

Adult Swiss albino mice in groups of ten were used in each test. Exposure periods ranged from 1-hr to 24-hr tests. The number of mice fatalities and the time of their death were recorded as an index of toxicity. The animals that died in each test were necropsied and grossly examined. Tissues from the first and last to die in each individual exposure were preserved in paraffin for use in the preparation of microscopic slides for detailed study.

Experiments were completed with combinations of oxygen, carbon monoxide, and carbon dioxide of various temperature levels. Analysis of the data indicates the significance of these variables when encountered in combination of two or three at a time as opposed to exposures of one or two at a time. Additional tests incorporating the use of selected noxious gases, namely, sulfur dioxide, nitrogen dioxide, and hydrogen cyanide, indicated the extreme toxicity of these variables when encountered in trace quantities superimposed on the previously studied variables.

Extension of the laboratory experiments was completed by generating actual "combustion products" from the burning of various building materials, chiefly Douglas-fir. The results of animal exposures to these combustion products were then compared with the results of the previous exposures without the presence of smoke and all of the trace elements present. Again, an increase in the overall toxicity was found. In conjunction with these exposures, a limited amount of qualitative and quantitative analyses were conducted for the purpose of defining and controlling the exposure conditions.

Gross examinations of the animals killed did not reveal any lesions however, generalized congestion was found. Histopathological examination of the prepared slides indicated that the degree of congestion found was similar regardless of the makeup of noxious gases or smoke evaluated. The congestion and absence of lesions are the typical findings when death is due to heat prostration and/or heat and anoxia.

The relationship between individual physical activity and fatalities under the exposure conditions was also examined. In a portion of the tests conducted, the physical activity levels of the animals were recorded at 5-min intervals by means of a relative activity "score." Comparison of these "scores" indicates a higher activity level for those mice dying at any one time as compared to the activity "scores" for the survivors; however, the difference noted was not felt to be of sufficient degree to be conclusive.

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## I. INTRODUCTION

### A. Background

#### 1. General

Previous studies of mass fires have indicated their importance as a military weapon. (1, 2)\* Mass fires created in the cities of Tokyo, Dresden, and Hamburg during World War II are still being studied because of the high death tolls involved. (3-7) It is well recognized that these fires were created through the use of conventional high explosive and incendiary bombs. With the advent of nuclear weapons and the use of high energy, high altitude, maximum thermal pulse delivery, the threat of fire as a weapon of war is even more significant. (8, 9)

The primary hazard to life in mass fires has been identified as exposure to the combustion products from the burning of various building materials. Beyond this simple conjecture, little can be said. The relative importance of the primary elements of combustion products is not known to a degree permitting consideration in the design of shelters. The importance of certain individual constituents has been studied and information regarding human exposure limits is available. (10) However, the relative importance of human exposure to one variable and human exposure to a combination of variables in the fire environment is little understood.

#### 2. Primary Hazards

Previous investigators have considered this question and several literature survey reports as well as a few limited experimental programs have been conducted. (11-13) In general, it has been concluded that the variables of carbon monoxide, anoxia, and hyperthermia are the "... recognized factors of primary significance ..." on the basis of exposures to single variables. Which variables are important in exposure to combinations of the constituents as found in combustion products is not known.

Thus, the life safety threat posed by mass fires in an urban environment is unclear. With the possible occurrence of mass fire in our cities during conflict, it is recognized that personnel in civil defense shelters or elsewhere within the involved area could be exposed to various concentrations of a variety of combustion products. Further definition of the hazard presented by exposure to such an environment will be of assistance to civil defense planners by providing information that can be used in evaluating the effectiveness of any shelter program.

---

\*Superscript numbers in parentheses refer to the List of References, Section IX.

### 3. Previous Studies

In a continuing program sponsored by the Office of Civil Defense, Southwest Research Institute has completed an initial study which defined the elements and placed them in perspective.<sup>(2)</sup> From this study evolved an overall plan which would allow advancement of the state-of-the-art, through a series of animal exposures, with regard to the definition of life hazard from combustion products.

A following study established controls with a series of exposures of white mice to single variables and to two and three variables combined.<sup>(14)</sup> From this study, the significance of combined exposures was demonstrated with clear synergistic results. A table was developed presenting estimated equivalence levels for humans and is included here as Table 1.

This current study was a follow-on to the previous programs and an effort to evaluate the significance of smoke and other noxious gases.

#### B. Scope of Work

##### 1. General

As outlined in Contract Order No. N0022868C 2721, Office of Civil Defense Work Unit 2537B, the specific objective of this work has been "to define and determine the relative importance and the synergistic effects of the hazards to life in a mass fire environment."

As contained in Paragraph 2.5, Scope of Work of the Award/Contract, the work to be performed included but was not limited to the following:

- "(1) Conduction of experimental studies using carefully controlled animal tests, to assess the relative importance of smoke and noxious gases other than carbon monoxide and carbon dioxide as casualty producers, individually and in combination with each other and with other previously investigated hazards, including carbon monoxide, carbon dioxide, heat, and anoxia. Among the noxious gases, the effects of which will be investigated are: sulphur dioxide, hydrogen chloride, nitrous oxide, hydrogen cyanide and phosgene.
- (2) Histopathological studies of the respiratory tract and vital organs of a representative selection of the animals used in the tests, to determine the nature and extent of injury.

TABLE 1. LEVELS OF SINGLE AND COMBINED VARIABLES  
CAUSING DEATH IN 4 HR<sup>(4)</sup>

Variables	Lethal Levels for Mice (for 4-hr exposure)	Estimated Lethal Levels for Humans (for 4-hr exposure)
<b>Single Variable</b>		
T* - Hyperthermia	100°F	130°F
O <sub>2</sub> - Anoxia	7.5% O <sub>2</sub>	8% O <sub>2</sub>
CO	0.125% CO	0.04% CO
CO <sub>2</sub>	40% CO <sub>2</sub>	20% CO <sub>2</sub>
<b>Two Variables</b>		
O <sub>2</sub> (Anoxia) + T	16% O <sub>2</sub> + 100°F 7.5% O <sub>2</sub> + 90°F	17% O <sub>2</sub> + 130°F 8% O <sub>2</sub> + 110°F
CO + T	0.075% CO + 95°F	0.02% CO + 120°F
CO <sub>2</sub> + T	30% CO <sub>2</sub> + 95°F	14% CO <sub>2</sub> + 120°F
O <sub>2</sub> + CO	16% O <sub>2</sub> + 0.075% CO 13.5% O <sub>2</sub> + 0.050% CO	17% O <sub>2</sub> + 0.02% CO 14% O <sub>2</sub> + 0.01% CO
O <sub>2</sub> + CO <sub>2</sub>	13.5% O <sub>2</sub> + 30% CO <sub>2</sub>	14% O <sub>2</sub> + 14% CO <sub>2</sub>
CO + CO <sub>2</sub>	0.075% CO + 30% CO <sub>2</sub>	0.02% CO + 14% CO <sub>2</sub>
<b>Three Variables</b>		
O <sub>2</sub> + CO + CO <sub>2</sub>	13.5% O <sub>2</sub> + 0.05% CO + 5% CO <sub>2</sub> 16% O <sub>2</sub> + 0.05% CO + 30% CO <sub>2</sub>	14% O <sub>2</sub> + 0.01% CO + 5% CO <sub>2</sub> 17% O <sub>2</sub> + 0.01% CO + 14% CO <sub>2</sub>
O <sub>2</sub> + CO <sub>2</sub> + T	11% O <sub>2</sub> + 5% CO <sub>2</sub> + 95°F 13.5% O <sub>2</sub> + 10% CO <sub>2</sub> + 95°F 16% O <sub>2</sub> + 20% CO <sub>2</sub> + 95°F	11% O <sub>2</sub> + 5% CO <sub>2</sub> + 120°F 14% O <sub>2</sub> + 7% CO <sub>2</sub> + 120°F 17% O <sub>2</sub> + 10% CO <sub>2</sub> + 120°F
CO + CO <sub>2</sub> + T	0.05% CO + 5% CO <sub>2</sub> + 95°F 0.05% CO + 20% CO <sub>2</sub> + 90°F 0.075% CO + 10% CO <sub>2</sub> + 90°F	0.01% CO + 5% CO <sub>2</sub> + 120°F 0.01% CO + 10% CO <sub>2</sub> + 110°F 0.02% CO + 7% CO <sub>2</sub> + 110°F
O <sub>2</sub> + CO + T	16% O <sub>2</sub> + 0.05% CO + 90°F	17% O <sub>2</sub> + 0.01% CO + 110°F

\*Temperature

- (3) Conduction of necropsies of a representative selection of the animals used in the tests, including examination of vital organs (heart, lung, liver, kidney, brain) for gross pathological changes.
- (4) An attempt to establish the pertinence of the results of these experiments to the problem of prediction of injuries that might occur to human beings in a mass fire environment."

The studies reported herein were designed and completed to evaluate, by means of carefully controlled animal tests, the life safety hazard presented by various combinations of carbon monoxide, anoxia, hyperthermia and carbon dioxide, sulfur dioxide, nitrogen dioxide, and hydrogen cyanide. Exposures were completed with animals utilizing combustion products which included smoke.

Necropsies were performed on all animals killed and gross examinations completed. In addition, histopathological studies of the respiratory tract and vital organs were conducted to determine the nature and extent of injury. In conclusion, the significance of the experimental results is discussed in terms of their meaning with reference to humans.

## 2. Range of Variables

Combustion products from burning materials contain hundreds of complex but separately identifiable components. These combustion products may be considered as providing three basic types: smoke, heat, and noxious gases. Smoke is considered as the solid particulate matter suspended in the gaseous environment.

Following combustion, there may be several constituents which tend to condense as the temperature of the "smoke cloud" decreases. These condensables may concentrate on the thousands of particles of solid matter, or smoke as defined previously, providing yet another separately identifiable variable which is subject to ingestion by humans.

## 3. Primary Variables

With such a diversity of variables, it is difficult to select a manageable number for more detailed study to allow definition of their relative importance. As a starting point, previous studies have indicated four major variables which the preponderance of opinion suggests are the most significant.<sup>(2)</sup> These are carbon monoxide, anoxia, hyperthermia, and carbon dioxide. In fact, one study suggested that "... the chief hazards in this connection are due to the presence of carbon monoxide or atmospheres lacking in oxygen . . ."(12)



It is from this standpoint that the study was initiated with definition of the relative hazards of each component singly, followed by evaluation of their combined effects.

#### 4. Noxious Gases

Selection of one or more specific noxious gases other than carbon monoxide and carbon dioxide for study might imply to some that the selected noxious gases are the most hazardous. However, no reliable data have been found which could be regarded as defining the relative significance of any particular noxious gas in the fire environment. It was apparent that there was no particular technical validity in the selection of one or more specific noxious gases, other than those mentioned, on the basis of hazard. Therefore, five gases were selected on the basis of a review of analytical analyses of combustion products from various building materials, chiefly wood.

Those gases selected were nitrogen dioxide, sulfur dioxide, hydrogen chloride, hydrogen cyanide, and phosgene. These five were not considered particularly significant but were simply selected as a starting point for use in this first experimental study of a complex problem.

#### 5. Confined Effects

Previous work in this program has been accomplished with the use of an animal exposure chamber within which a particular gaseous environment was created. The animals were then inserted and their times of death recorded. It has been observed in previous work that all of the animals do not react in the same manner and that their physical activity is markedly different from animal to animal. To allow for evaluation of this variance as related to survival or time of collapse, a chamber was fabricated for this study which provided a separate compartment for each animal. This then allowed for identification of each animal and the observance and recording of its physical activity during each test. With such recorded data, it could be examined with respect to any correlation that might be indicated between physical activity and time of death.

#### 6. Mechanism of Death

Immediately following each test, the first and last animals to die were identified for histopathological examination. These animals were necropsied and a gross pathological examination conducted. Tissue was excised and preserved and placed on slides. Following completion of major segments of exposure tests, the tissues were examined by a veterinary pathologist and the nature and extent of injury identified.

## C. Approach

### 1. Laboratory Exposures

The basic approach utilized in this study has been to expose groups of white mice to carefully controlled mixtures of simulated and actual combustion products. Throughout the study, the results of each test were evaluated and this information utilized in the planning of subsequent exposures.

The test chambers, instrumentation, and gas mixing equipment utilized are discussed in Paragraphs II. A, II. B, and II. C. The test animals and the specific handling procedures are discussed in Paragraphs III. A and III. B.

Approximately ten variables have been considered in this study, including physical activity. Throughout the program, exposures were designed to evaluate the incremental contribution by the addition of one variable (or a group of variables) to others, the effect of which had been previously established.

### 2. Controls

Work initiated during an earlier phase of Work Unit 2537A was based upon a series of exposures to single variables (carbon monoxide, temperature, oxygen, carbon dioxide) to define minimum lethal concentrations under the test conditions.<sup>(14)</sup> With these data as a control, two variables were then combined for definition of a similar minimum lethal condition. The differences between the individual toxic concentrations and the combined concentrations were evaluated to provide a measure of the increased toxicity brought about by the combined variables. The results of these double exposures then served as controls for the addition of a third variable.

In this current program, the work has been extended to allow study of a few selected noxious gases and to include an evaluation of the importance of smoke. For this work, the results of the previous Work Unit provide a starting point and control.

### 3. Particular Variables

The variables included and discussed in this project include the following:

Primary Variables - carbon monoxide  
hyperthermia  
anoxia  
carbon dioxide

Noxious Gases - sulfur dioxide  
nitrogen dioxide  
hydrogen cyanide  
hydrogen chloride  
phosgene

Smoke (particulate matter)

Physical Activity

Sex

The monitoring and analysis of the primary variables, noxious gases, and humidity are fully explained in Section II, Paragraphs B and C. The procedure utilized in recording the physical activity of the animals is covered in Section III, B. 2.

#### 4. Acute and Chronic Effects

The results from these studies are intended to provide data to allow evaluation of the effects of combustion products on humans. The exposure conditions considered may be described as acute with incapacitating or lethal results in relatively short periods of time. Review of the literature indicates a serious lack of reliable data regarding the relative toxicity of acute human exposure to the variables of interest. Some data are available regarding longer chronic exposures and the recommended threshold levels for minimum physical response. (10)

To study the variables of interest in detail, including various combinations, it is necessary to expose these environments to a physiological system which will reflect the response in man. The use of mammalian species in toxicity testing is widely accepted. (15, 16) Some of those commonly used are the mouse, rat, dog, and monkey. In nearly all such studies, the mouse is used when a large number of tests and animals are required to define the toxic environment. It can be purchased from breeders in excellent health with uniform genetic characteristics. This species is also useful for this study because of the amount of background information available on the animal.

## II. TEST APPARATUS AND INSTRUMENTATION

### A. Test Chambers

In order to accurately determine the toxicity of various components of a fire atmosphere, an exposure chamber is required in which the environment can be accurately monitored and maintained. In a previous program (OCD Work Unit 2537A), a 1-cu-ft chamber was utilized.<sup>(14)</sup> During the present work unit, two new chambers have been designed and constructed which permit more accurate observations of individual animal's physical activity during the test program. They also provide a considerably larger amount of space for each animal, which is essential for accurately determining the toxicity of individual components. Crowding of animals may produce errors in toxicity testing. One of the chambers was designed to study the effects of various elements of a fire atmosphere artificially produced by bottled gases; thus, a flow-through system was provided to maintain the desired atmosphere throughout the test period. This chamber is hereafter referred to as the "flow-through chamber."

The second chamber was designed so that a sample of an actual smoke atmosphere could be entrapped, the animals inserted, and observed for a maximum of 4 hr. This chamber was sufficiently large so that the presence of ten mice would not appreciably alter the composition of the gases during a 4-hr exposure period. This chamber is hereafter referred to as the "static exposure chamber." Both of these chambers incorporated the capability to house the mice in individual compartments to facilitate activity measurements.

#### 1. Flow-Through Chamber

The flow-through chamber was constructed of clear, 1/4-in. plastic sheets. The interior chamber measured 35.2 in. wide X 17.2 in. deep X 11 in. high (inside dimensions) for a total interior volume of 650 cu in. (3.85 cu ft). Ten individual animal compartments were formed using an assembly of perforated galvanized metal sheets within the chamber. Each animal compartment had interior dimensions of 11.4 in. deep X 6 in. wide X 7.8 in. high, providing approximately 535 cu in. or 0.39 cu ft of space for each animal. The animal exposure chamber with individual animal compartments is shown in Figure 1. The chamber was fitted with three openings which permitted the insertion of mice into the individual compartments without undue interruption of the gaseous atmosphere. The chamber also had fittings and vents necessary to service the various sample lines and instrument connections to complete the system. These are fully described in Paragraphs II. B and II. C.

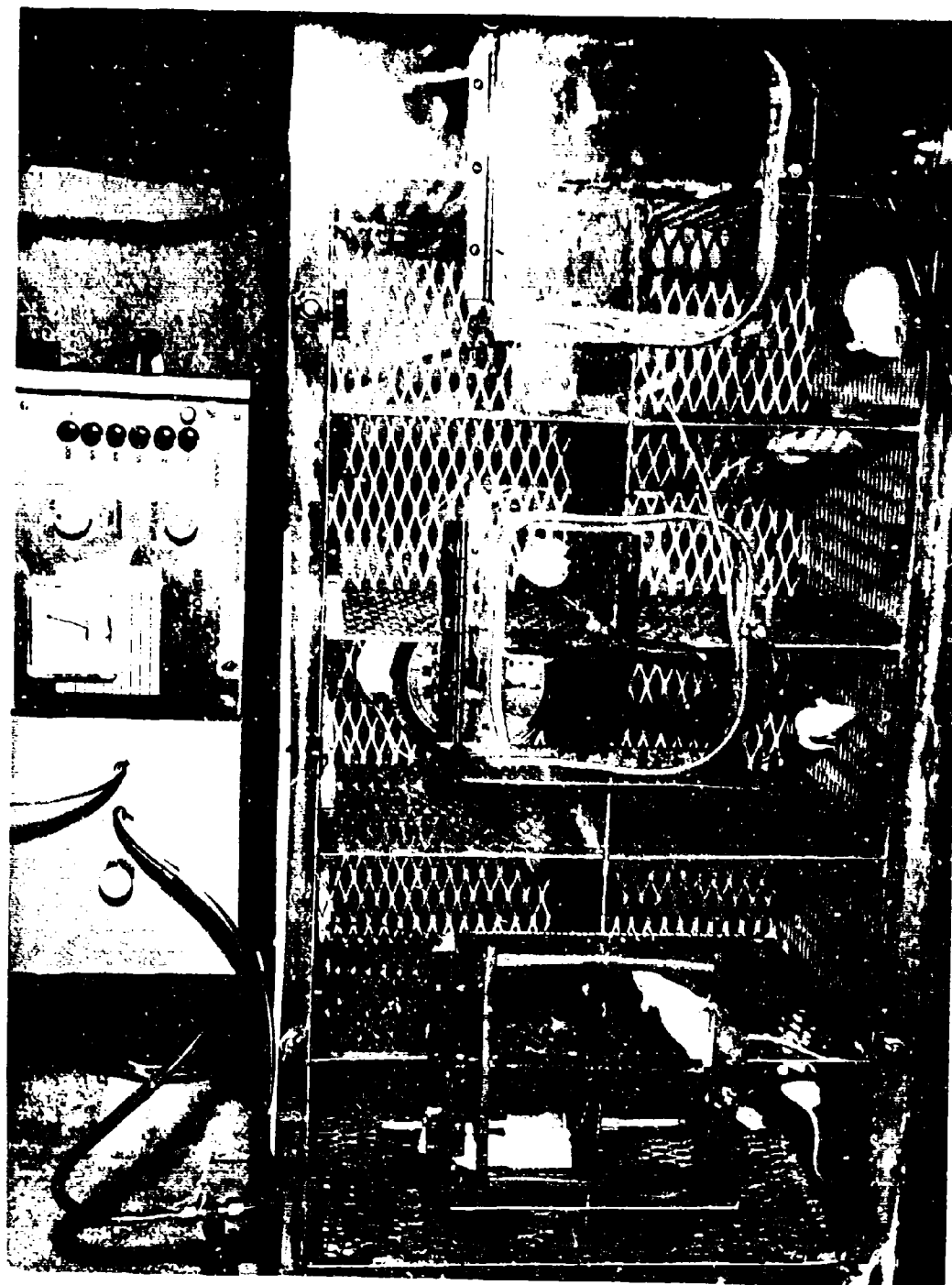


FIGURE 1. FLOW-THROUGH EXPOSURE CHAMBER  
WITH INDIVIDUAL COMPARTMENTS

## 2. Static Exposure Chamber

A box constructed of 1/4-in. plastic sheets with an interior volume of approximately 10 cu ft was used in this phase of the experiment, allowing an area of 1 cu ft per animal. This chamber measured 48-1/4 in. wide X 24-1/2 in. deep X 15-1/4 in. high. Ten stainless steel wire test tube baskets (5 X 4 X 5 in.) were used to hold each animal. These baskets were attached to the underside of the top of the box. Removable lids over the tops of the baskets provided a way of placing the animals in the baskets with minimal disturbance of the atmosphere in the box.

The smoke entered the box from a test furnace (see Section IV-B for details) through a 1-in. -ID pipe which extended 12 in. into the furnace. This had 3/8-in. perforations on one side to channel the smoke into the box. The combustion products were pulled into the box by suction through a 3/4-in. pipe attached to a vacuum pump located outside the box. Two cutoff valves were installed in the smoke input line, one after the smoke left the furnace and the other before the smoke entered the box. A third cutoff valve was installed between the vacuum cleaner and the box so that the smoke could be trapped in the box. Figure 2 shows a photograph of the chamber.

## B. Noxious Gas Mixing System

### 1. Equipment

To introduce the desired concentration (parts per million) of noxious gases such as hydrogen chloride, nitrogen dioxide, sulfur dioxide, and hydrogen cyanide, a different method than used previously was perfected. A schematic of the system is shown in Figure 3. The system described below was utilized in conjunction with the basic flow-through chamber.

The noxious gases, nitrogen dioxide, sulfur dioxide, and hydrogen chloride, were received in compressed lecture bottles. In order to maintain the desired concentrations of these gases individually, they were first mixed to a known concentration prior to introduction into the animal chamber. A 27,000-cc Nalgene aspirator carboy was used as the mixing chamber. A No. 13-1/2 rubber stopper was used to plug the top of this chamber. This contained two 1/4-in. holes (inlet and outlet) for tubing connection to a Neptune Dyna circulating pump (Model No. 2). Two additional 1/8-in. holes in the stopper were provided to connect the high pressure compressed air cylinder through a Mathison Company, Inc., R-2-15-AAA flowmeter to the bottom of the mixing chamber with 1/8-in. Teflon® tubing. A second 1/8-in. Teflon® tubing line from the top of the mixing chamber connected it to the animal chamber. Before the desired amount of concentrated noxious gas was introduced into the mixing chamber, a slight vacuum was placed in the chamber with the use of a glass syringe. A gas injection port on the stopper was available with a three-way Teflon® stopcock attached

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\*DuPont registered trademark.

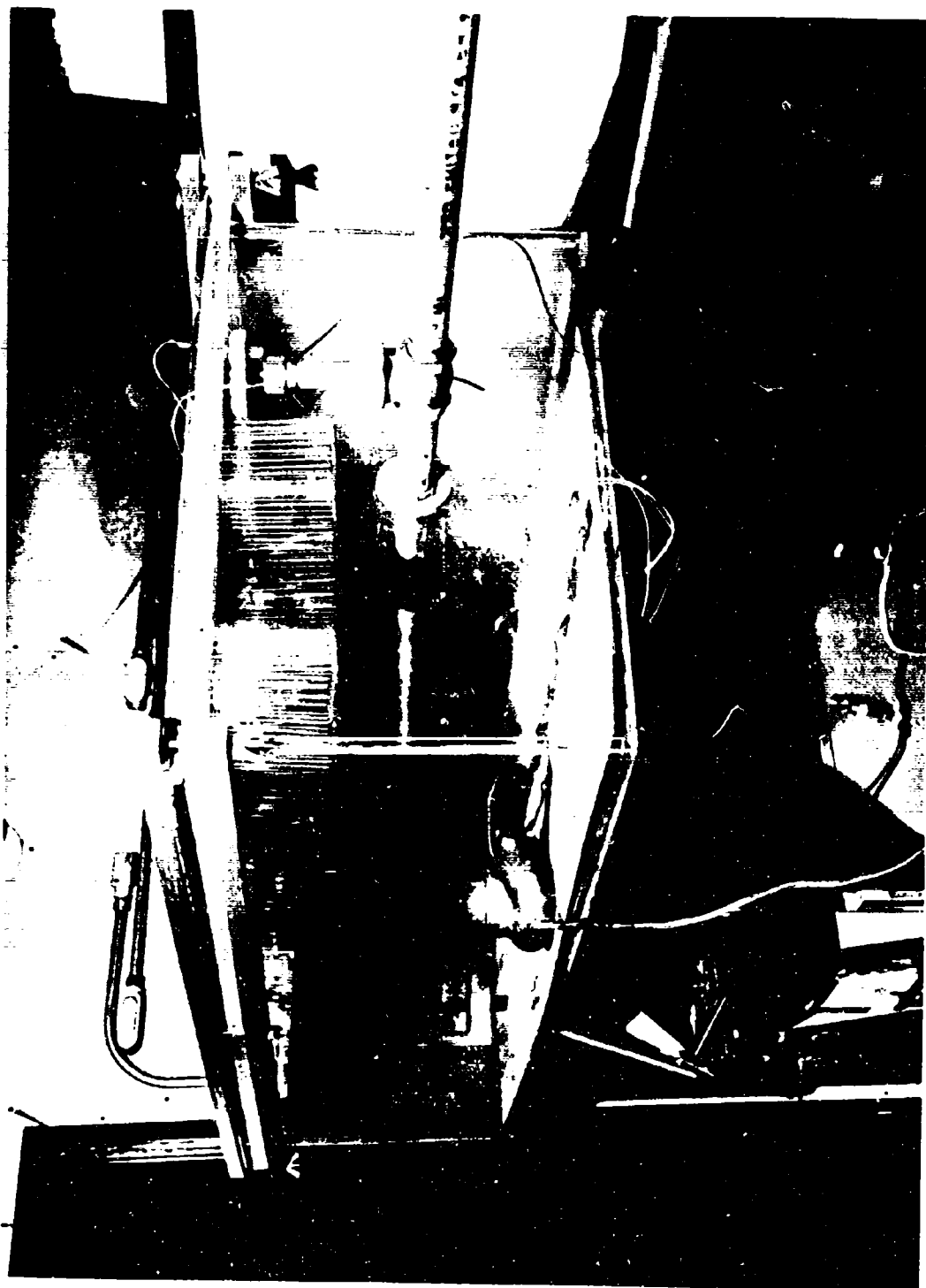


FIGURE 2. STATIC EXPOSURE CHAMBER

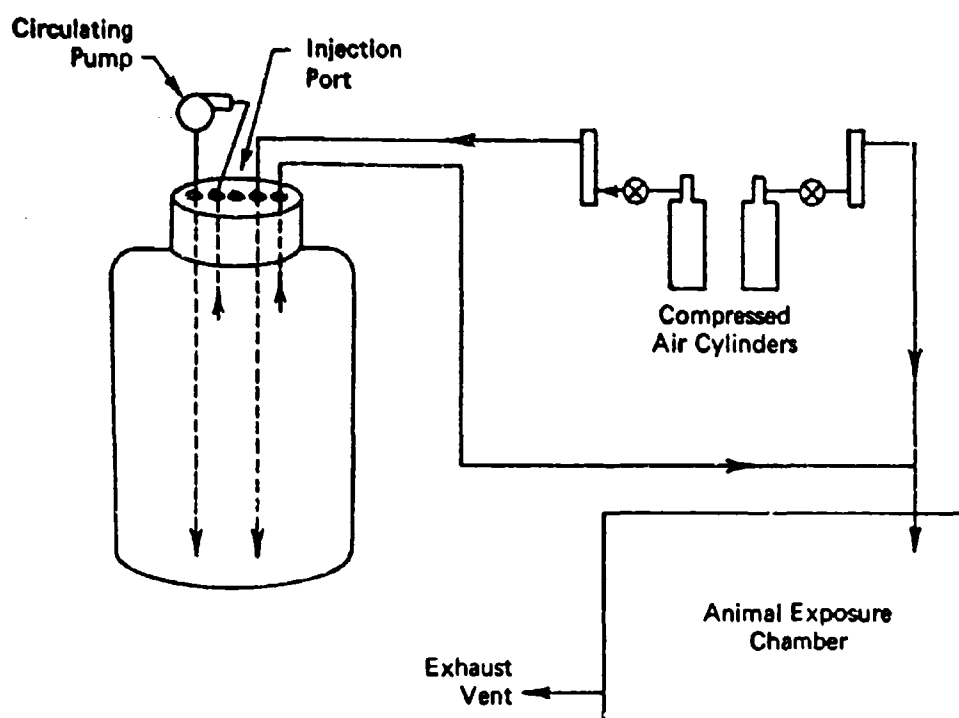


FIGURE 3. NOXIOUS GAS MIXING ARRANGEMENT



to a 3-in., 16-gauge stainless steel needle through which the precise amount of noxious gas could be added.

## 2. Operation

The gas was mixed by the circulating pump for 10 to 15 min before it was introduced into the animal chamber. The flow through the mixing chamber was derived by:

$$K_{ac} = \frac{F_{mc}K_{mc}}{F_{ac}}$$

where:

$K_{ac}$  = desired concentration of noxious gas in animal chamber

$K_{mc}$  = concentration of noxious gas in mixing chamber

$F_{ac}$  = total flow into animal chamber

$F_{mc}$  = total flow through mixing chamber

The animal chamber was monitored periodically to determine the gas concentrations in order to check the validity of the above system. With gradual depletion of the noxious gas in the mixing chamber with time, concentrated amounts had to be added hourly.

Hydrogen cyanide was handled differently. This gas was mixed (1.9 percent) with compressed air in a high pressure cylinder. It was then introduced through a flowmeter directly into the animal chamber, after the appropriate dilution with compressed air. The mixing chamber was not used in this case. Due to the extremely hazardous conditions in handling hydrogen cyanide, maximum safety precautions were used at all times. Two experimenters were always on hand and both used complete SCUBA diving gear, which included harnessed compressed air cylinders with eye-nose masks. This equipment was worn while near the experimental animal chamber and also while in the laboratory.

## C. Instrumentation

To establish the toxicity of various components of a fire atmosphere to animals, it was essential that the environments in both exposure chambers be accurately known and controlled. The instrumentation required for maintaining these environments was similar for both exposure chambers used in this study. The major difference in the two chambers was that the basic chamber was a flow-through system requiring constant flow with the metering equipment and instrumentation necessary for maintaining the desired atmosphere.

In the static exposure chamber, an atmosphere was entrapped in the chamber and maintained for the duration of the test. In both exposure chambers, an accurate control of temperature was essential. The humidity was not controlled; however, it was monitored in both chambers.

1. Temperature Control

The temperature on both exposure chambers was controlled using electric heating tapes combined with Foxboro temperature indicators and a Thermistemp temperature controller. A blower was used to insure that proper circulation was maintained throughout each chamber. The basic (flow-through) chamber had a Dayton 1/100-hp, 1520-rpm motor with a 4-in. blade and a 3-in. shaft. The static chamber utilized a Model 22075 blower/heat gun manufactured by Dayton Electric Manufacturing Company.

2. Humidity

The relative humidity was monitored by means of an Abbeon relative humidity gauge.

3. Gas Flow

To maintain the proper flow of gases in the flow-through system, an accurate metering of oxygen, carbon dioxide, and carbon monoxide was essential. A schematic of the flow-through exposure chamber is shown in Figure 4. To obtain the desired concentration of oxygen, carbon dioxide, and carbon monoxide in the flow-through chamber, the flowmeters were initially calibrated and used as a means of establishing the proper mixture. Once this flow was set, a more accurate gas analysis was provided with analytical instruments to indicate the actual gas concentration throughout the test chamber. Necessary corrections in the precise concentration were then made using manual adjustment of the flowmeters. For metering very small quantities of gases such as the noxious gases, a gas mixing system was used as described in II-B. With that system, concentrations of gases were introduced in the chamber which required a considerable dilution prior to the metering of the gas mixture into the exposure chamber.

When the gas flows were initiated, the heater and fan were turned on and the temperature setting made. This setting was allowed to equilibrate at the proper level before precise setting of the individual gas flows was made. After the initial setting of the gas flow, a period of 10 to 15 min was allowed to elapse before starting the test for the animals to insure proper equilibration. The test animals were then put into the compartments through the transparent plastic doors on the front of the test chamber.

The test chamber was maintained under a hood vented to the outside of the building. A total flow of 4 l/min was established and held

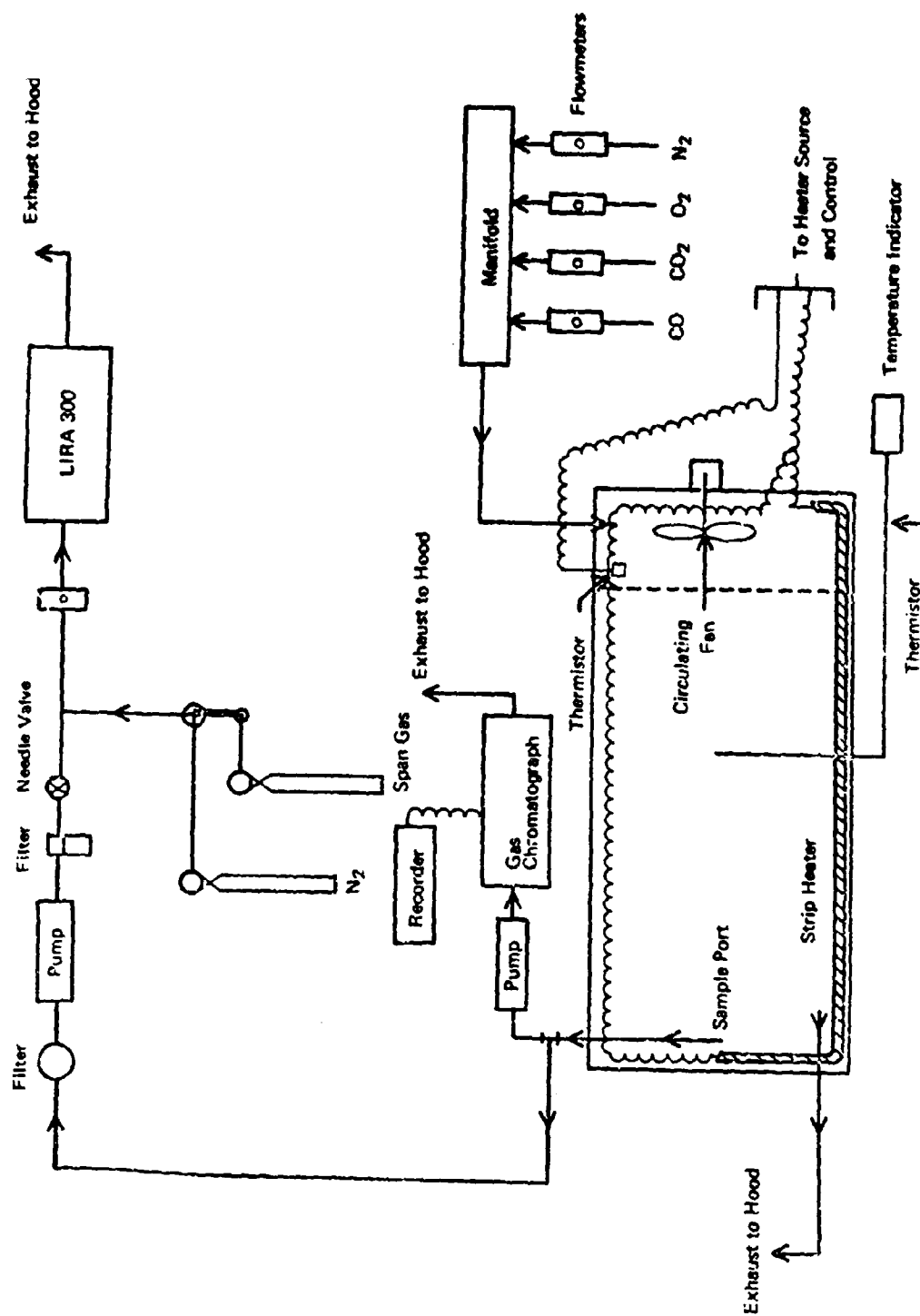


FIGURE 4. SCHEMATIC OF FLOW-THROUGH EXPOSURE CHAMBER

constant through all tests except the static chamber experiments. The 4-l/min exhaust gases were vented to the atmosphere outside of the building. This was a single-pass system. Each test was run with a balance gas of nitrogen rather than room air. This was done to eliminate the necessity of adding nitrogen to the chamber to obtain conditions of anoxia. Those tests which required normal atmospheric conditions, that is, 21 percent oxygen, were achieved through the use of high pressure tanks of oxygen and nitrogen (21 percent oxygen, 79 percent nitrogen).

#### 4. Gas Analysis

##### a. Flow-Through Chamber

A Fisher Gas Partitioner (gas chromatograph) was used to monitor the concentrations of gases in the test chamber. This was equipped with thermal detectors. Gas separations were accomplished on two columns in series. The first column, 4 ft of 1/4-in. aluminum tubing packed with Porapak Q, separated carbon dioxide from the other gases. The second column, 15 ft of 3/16-in. copper packed with 13X molecular sieve, separated the nitrogen, oxygen, and carbon monoxide. This instrument was calibrated against concentrations of the gases in room air and known synthetic mixtures. Gas samples were introduced through a sample loop that insured the measurement of the same volume each time. Detector output was read on a Texas Instruments Servo/riter recorder, 0 to 1 mV full scale. Also, a model 52 YSI oxygen meter was attached to the animal chamber for periodic monitoring of oxygen.

Carbon monoxide levels were monitored visually on a low-flow flowmeter and were periodically checked by the gas chromatograph. Carbon monoxide levels were also constantly monitored by an M-S-A Lira Infrared Analyzer Model 300 to give accurate readings at low concentrations. The noxious gases, nitrogen dioxide, sulfur dioxide, hydrogen cyanide, and hydrogen chloride were detected and quantitated by M-S-A (Mine Safety Appliances) and Unico 400 (Union Environmental Instruments, Inc.) detector tubes (Figure 5). Calibration charts were provided by M-S-A and Unico for the various tubes.

##### b. Static Exposure Chamber

A closed system manifold (Figure 7) with a Beckman Z-300 diaphragm pump, a Beckman carbon dioxide (0- to 10-percent and 0- to 50-percent range) IR analyzer, a Beckman carbon monoxide (0- to 2-percent and 0- to 10-percent range) IR analyzer, and a Beckman Model F3 oxygen meter were used for constant monitoring of the gaseous atmosphere (Figure 8). These detecting instruments were attached to a Honeywell 12-point recorder. Also included in the closed system was the Lira Model 300 carbon monoxide

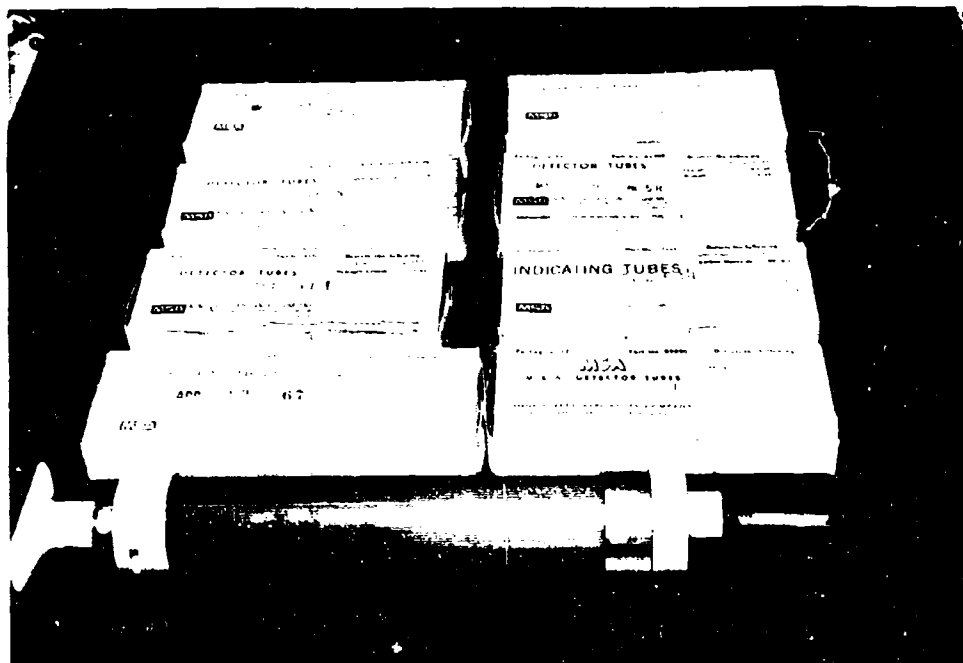
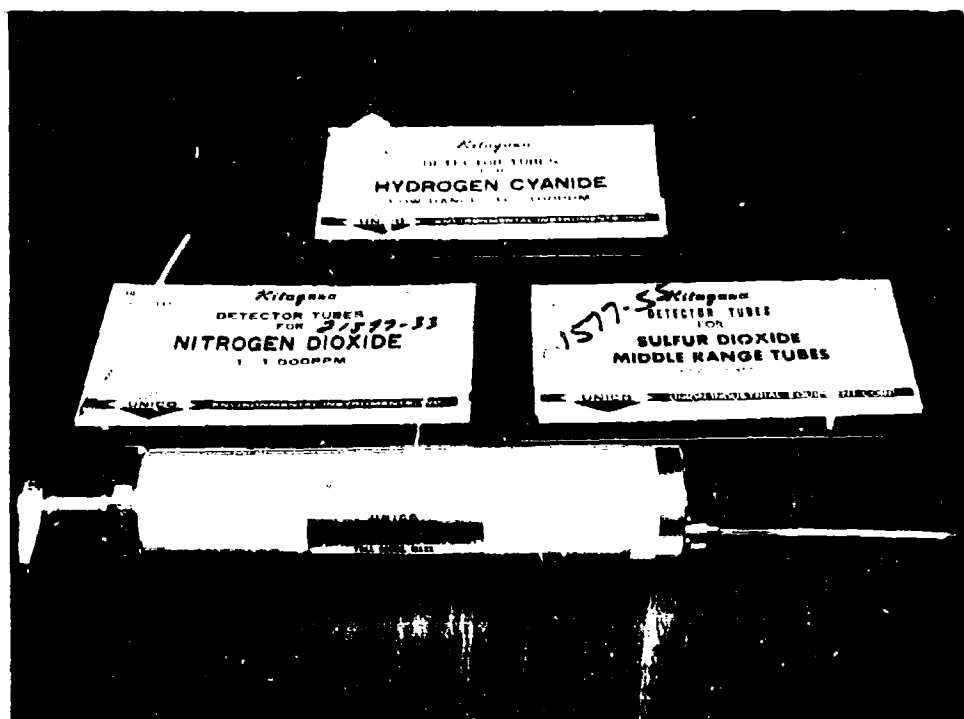


FIGURE 5. MSA AND UNICO COLORIMETRIC TUBES AND PUMPS

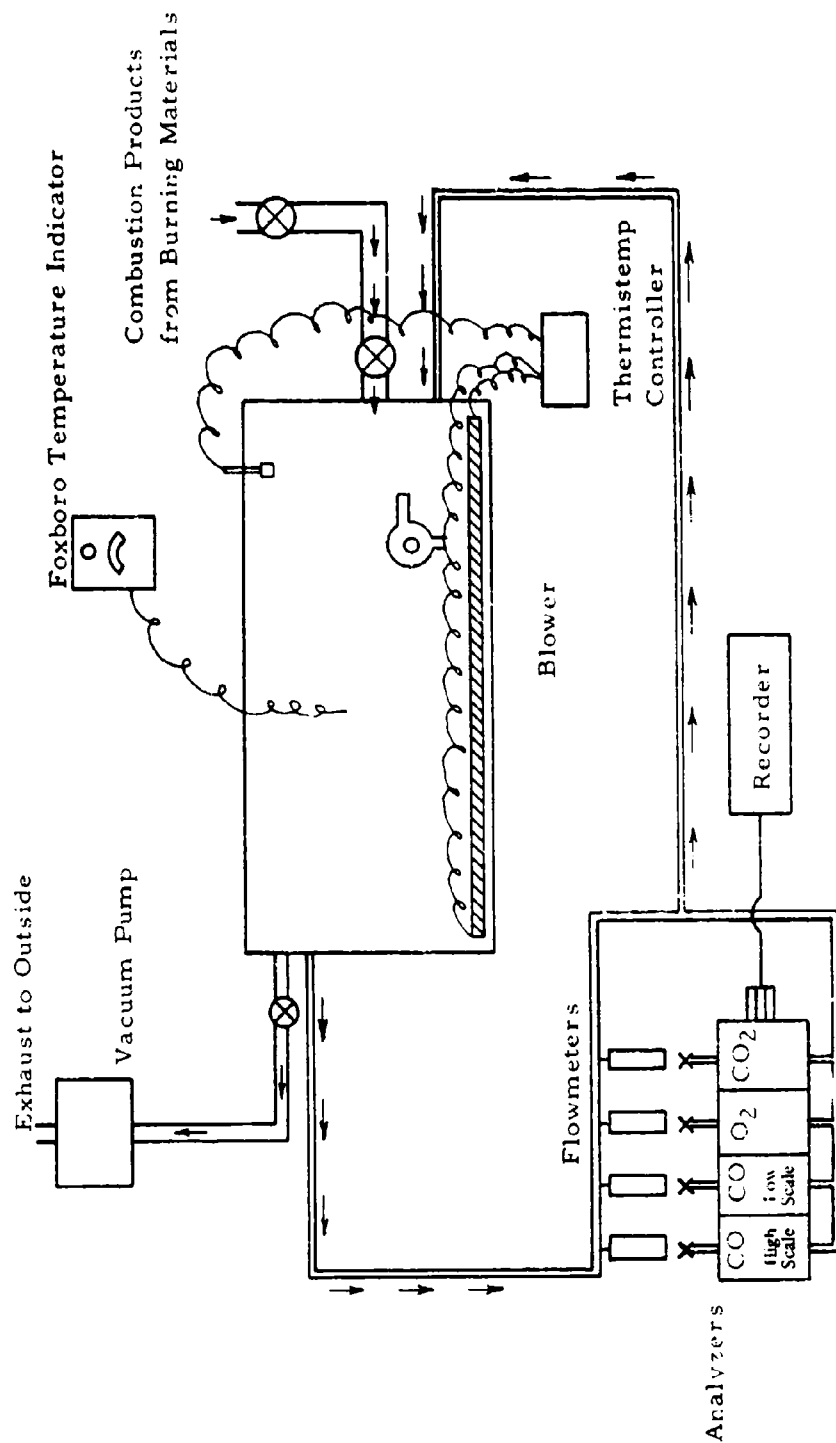


FIGURE 6. SYSTEM FOR MONITORING FIRE GAS  
IN STATIC EXPOSURE CHAMBER

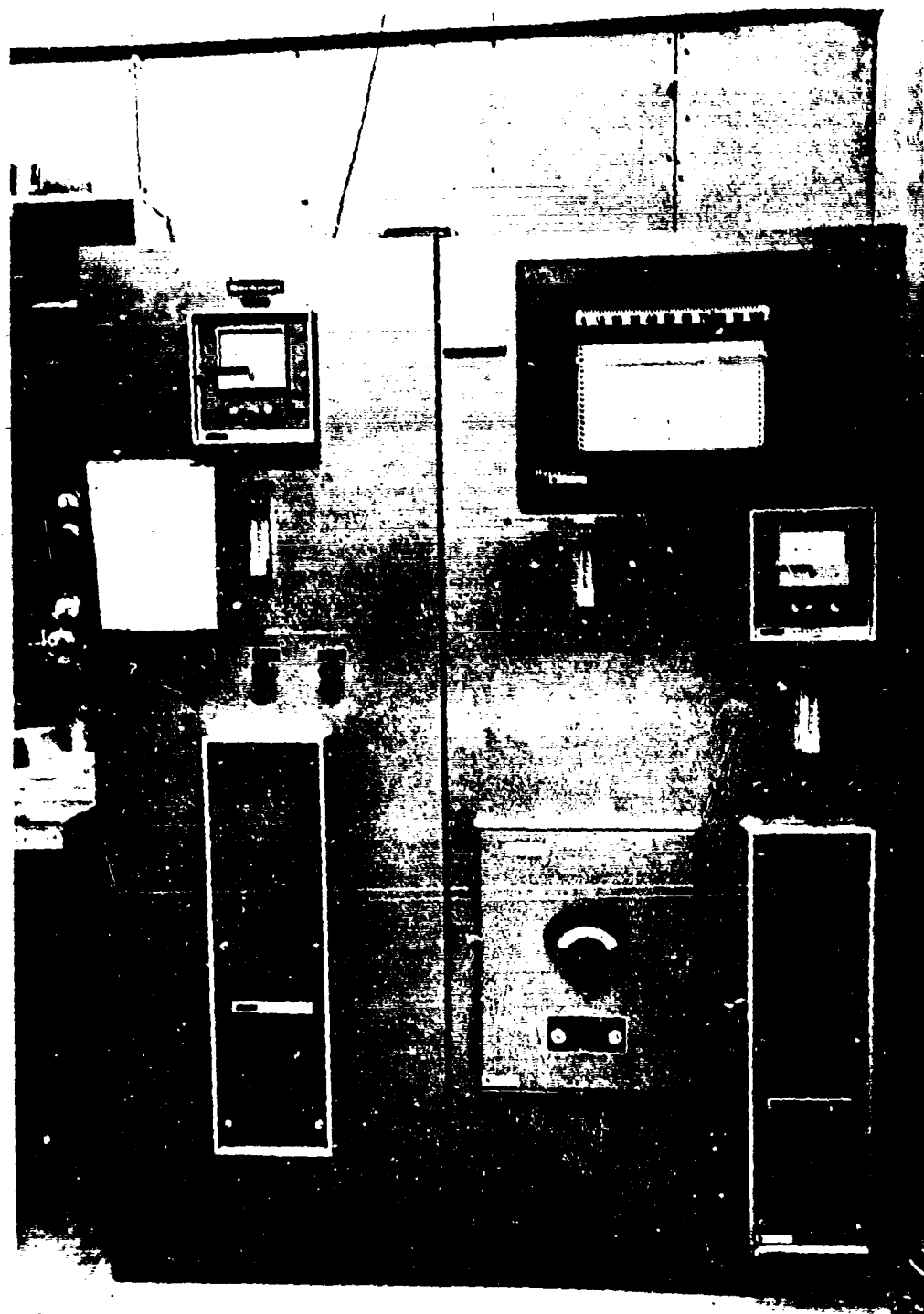


FIGURE 7. NOXIOUS GAS SAMPLING FROM STATIC  
EXPOSURE CHAMBER

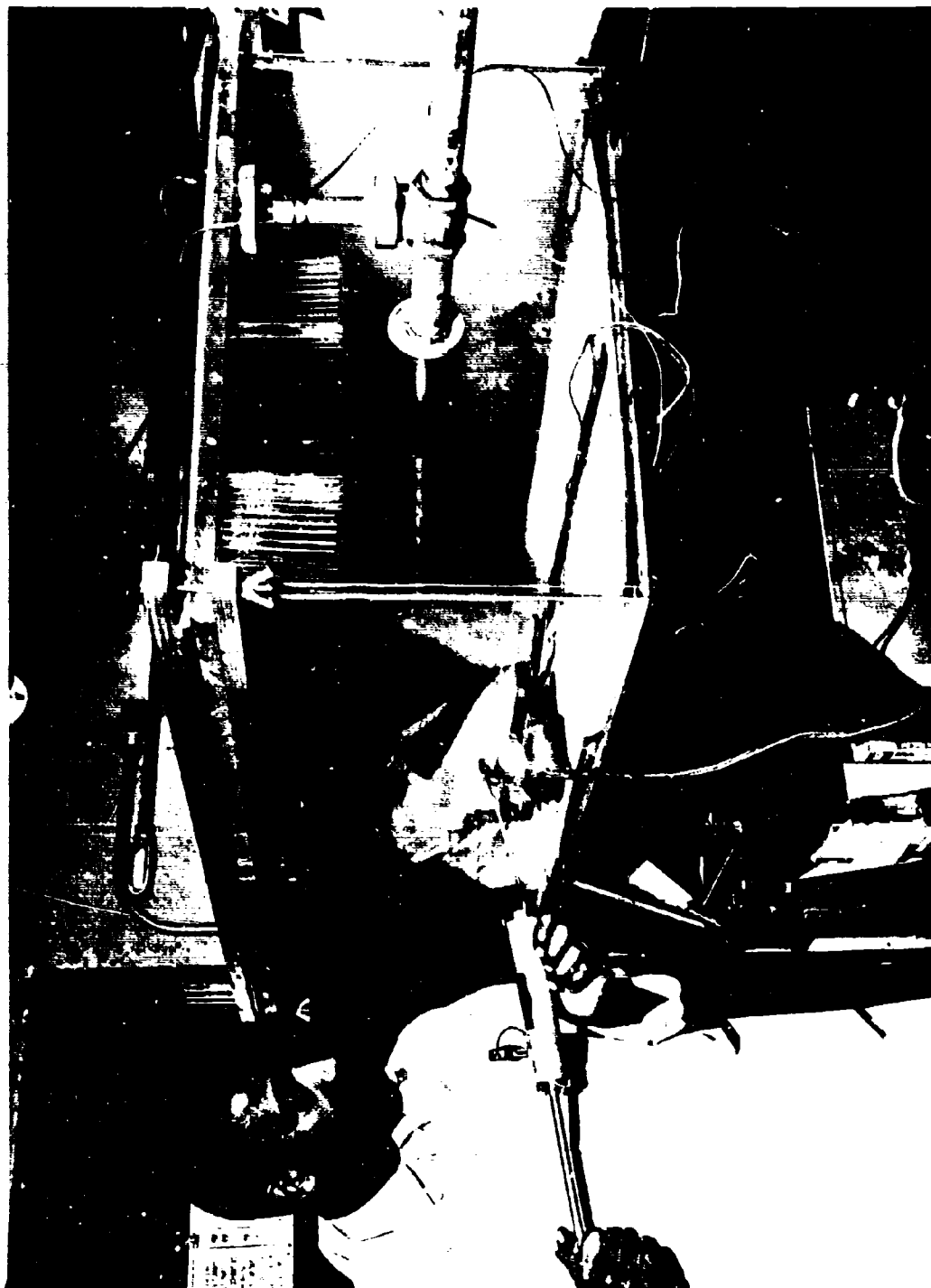


FIGURE 8. GAS ANALYSIS EQUIPMENT



analyzer. A 1/4-in. sampling port was used to insert the M-S-A and Unico sampling tubes for detection of other noxious gases within the chamber (Figure 8). The mice were put into their respective baskets after the controlled atmosphere was attained.

### III. TEST ANIMALS AND PROCEDURES

#### A. Test Animals

##### 1. Type

The mice used in this program were male Swiss albinos, Webster strain, random bred, from Simonson Farms, Gilroy California. These animals were shipped by Air Express and placed in isolation after arrival. Young male and female adults were generally used in this and previous programs.<sup>(2,14)</sup> The animals were ordered in batches of 400 to 500 in order to maintain approximately the same age of animals throughout the research program. The selected species was utilized because of its size and the economics in such a program where so many animals are required.

##### 2. Handling

The animals were housed five to a cage in standard laboratory cages with food and water fed ad libitum. Cages were cleaned twice weekly. Each animal was housed as described for a minimum of 10 days prior to experimental use. Any animals which appeared to be in poor health were removed from further consideration. Animals that survived the experimental testing were returned to their cages and observed for 10 days for abnormal signs and symptoms resulting from the exposure. After this post-test period, the animals were euthanized and some were grossly examined. Any animals that died during this 10-day period were listed as deaths for the experimental exposure time.

The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965, prepared by the Committee on the Guide for Laboratory Animals Resources, National Academy of Sciences--National Research Council; the regulation and standards prepared by the Department of Agriculture; and Public Law 89-544, "Laboratory Animal Welfare Act," August 24, 1967.

#### B. Test Procedures

##### 1. Chamber Conditioning

Whenever the basic variables--heat, anoxia, carbon monoxide, and carbon dioxide--were being evaluated in the flow-through chamber, the animals were placed into the chamber immediately after the conditions of the test were established through the transparent plastic doors on the front. These doors were fastened to the chamber with wing nuts which, when tightened, formed an airtight seal by pressing the door against rubber tubing placed around the opening. Insertion of the animals did not significantly affect the concentrations of gas within the chamber, and the conditions were

rapidly equilibrated. The chamber utilized in exposures of the various noxious gases did not contain the compartmented unit. It was found that this equipment absorbed the parts per million of the noxious gases.

In the study with combustion products, the gases plus the smoke were drawn in the static animal exposure chamber, analyzed, and adjusted to the desired level. The animals were then inserted into their individual cages through the ten different openings on top of the chamber and the chamber was sealed for the remainder of the test exposure.

## 2. Observation of Test Animals

For the study of single and combined exposures of the primary variables, the compartments of the flow-through test chamber were numbered 1 through 10 and one animal was placed in each compartment. All odd-numbered compartments housed the male mice for the duration of each test and the even numbers, the females. The animals were under constant surveillance throughout each test.

For the purpose of this program, the activity of each test animal was recorded at 5-min intervals from the time the animals were put into the test chamber until they were removed. The activities of the animals were recorded as scores, using numbers from 0 through 8, as seen in Table 2. A score of 4 denoted normal activity, whereas scores above and below 4 denoted progressing hyperactivity and hypoactivity. This system was selected through evaluation and modification of methods described by Turner.<sup>(17)</sup>

At the close of the test period, the surviving animals were returned to their cages and observed for 10 days for abnormal signs and symptoms resulting from the exposure. After this post-test period, the animals were euthanized. Time of death of the animals was determined by respiratory failure and loss of heartbeat. For purposes of this study, toxicity, mortality, and time of death were recognized as the indicators of hazard based on maximum exposure periods up to 24 hr. The tests involving the laboratory mixture of noxious gases or other combustion products (heat and smoke) did not use the activity measurements but the animals were observed for the entire experiment. Time of death was recorded and notes were kept concerning general activity level of the animals.

## 3. Test Duration

Most of the previous studies<sup>(14)</sup> were run using 4-hr exposures; however, during this past contract year, the time was extended in some exposures to 24 hr. These experiments were undertaken to provide a time-concentration sensitivity curve for the mice for the four primary

TABLE 2. SCORES USED TO DENOTE ANIMAL ACTIVITY

<u>Score</u>	<u>Activity</u>
0	Dead
1	Sleep
2	Still
3	Occasional motion
4	Constant walking and excessive inquisitive action
5	Walking with some running
6	Agitated spurts
7	Tremors
8	Convulsions

variables and for the additional noxious gases studied. The animals were under observation for the 24-hr period; however, detailed activity measurements were not made.

#### 4. Pathological Examinations

The dead animals from each test were examined grossly for signs of tracheal damage. The heart, liver, spleen, kidneys, lungs, and trachea were removed from selected animals from each test for detailed histopathological examination. These organs were preserved in formalin and later blocked in paraffin for storage and/or slide preparation. A total of 76 animals have been examined in detail using standard histopathological procedures.

#### IV. COMBUSTION PRODUCT DEFINITION

##### A. General

###### 1. Scope of the Problem

The subject of combustion products from the burning of building materials as found in the fire environment has long been of interest with relation to life safety. Previous studies have examined this subject from several aspects including definition of the various constituents to be found when particular materials are burned. Other studies include those which may be classified as reviews in cataloging much of the information. Some of these studies may be considered as state-of-the-art reviews.

In general, these studies may be grouped into three major categories: first, those that are primarily concerned with the identification and cataloging of identifiable elements found in the environment of interest; (11, 12, 18, 19) second, those that have concerned themselves with the definition of human toxicity to the many variables on an individual basis; (10, 20 to 28) and thirdly, those studies that have addressed themselves to the matter of exposure to the various elements as found in combinations under actual burning conditions. (2, 14, 29 to 32) Each of these studies, while having some relationship with the purposes of this program, provides limited information for input purposes. From previous review of these studies, applicable information was obtained and utilized in the formation of the overall program for this study.

###### 2. Needs of Present Program

Because there are so many variables, it was essential that all but a manageable number be eliminated from consideration. This restriction by itself raises questions. What significance is there in the elimination of any variables? In this regard, there is some guidance in the judgement of previous investigators who have suspected the importance of a few primary variables. However, even the acceptance of this as a valid conclusion might lead to erroneous results, since these conclusions were based upon work that did not include the type of experimental program considered essential to the assembly of supportive data.

Nonetheless, a starting point must be selected, and this selection requires the elimination of other "points." Accordingly, the best judgements of previous investigators were given full consideration in the design of this study and incorporated to various degrees. The major inclusion in this regard is the selection of carbon monoxide, heat, anoxia, and carbon dioxide as primary variables.

Beyond consideration of the aforementioned variables, little has been accomplished to define the relative significance of the many hundreds of other variables broadly classified as noxious gases and smoke. Areas where further study is necessary as well as those areas which may be regarded as relatively insignificant must be identified.

Therefore, the present program includes an initial effort to examine the relative importance (toxicity) of a few selected noxious gases found in the fire environment in addition to the four basic gases previously studied. The needs of the program are to define those noxious gases which are most significant with respect to life safety and to allow analysis of their relative importance with respect to injury and death.

## B. Experimental Work

To provide input data for the definition of noxious gases in this study, a series of tests was completed in which various building materials were burned and their combustion products analyzed both qualitatively and quantitatively.

### 1. Exposure Conditions

The building materials utilized in this phase of the study were burned in the ASTM E84 Flame Spread Tunnel Furnace located at Southwest Research Institute. (33)

This tunnel furnace, shown in Figure 9, basically consists of a 25-ft-long "box" with interior dimensions 12 ft high and 17 in. wide. This furnace or box is in the horizontal position and the material to be burned or exposed to the flame forms the top.

At one end of the tunnel furnace there is a 3-in. high, 17-in.-wide horizontal air inlet. There are also two gas burners at this end which provide the ignition flame. The other end of the tunnel furnace is connected to an exhaust plenum which provides a source of draft. During standard test operations, the exhaust system is adjusted to provide approximately 0.075 in. of draft at 360 CFM through the tunnel furnace at 240 lineal feet per minute.

In the course of this program, Douglas-fir wood was selected as a typical building material well suited for initial study purposes. Five tests were completed utilizing Douglas-fir, each under different and varying nonstandard ventilation conditions. Two tests were conducted with red oak flooring, one with a standard service grade vinyl asbestos tile, three with wool carpets, two with acrylic carpets, one with a polyester carpet, and one with PVC rigid pipe.

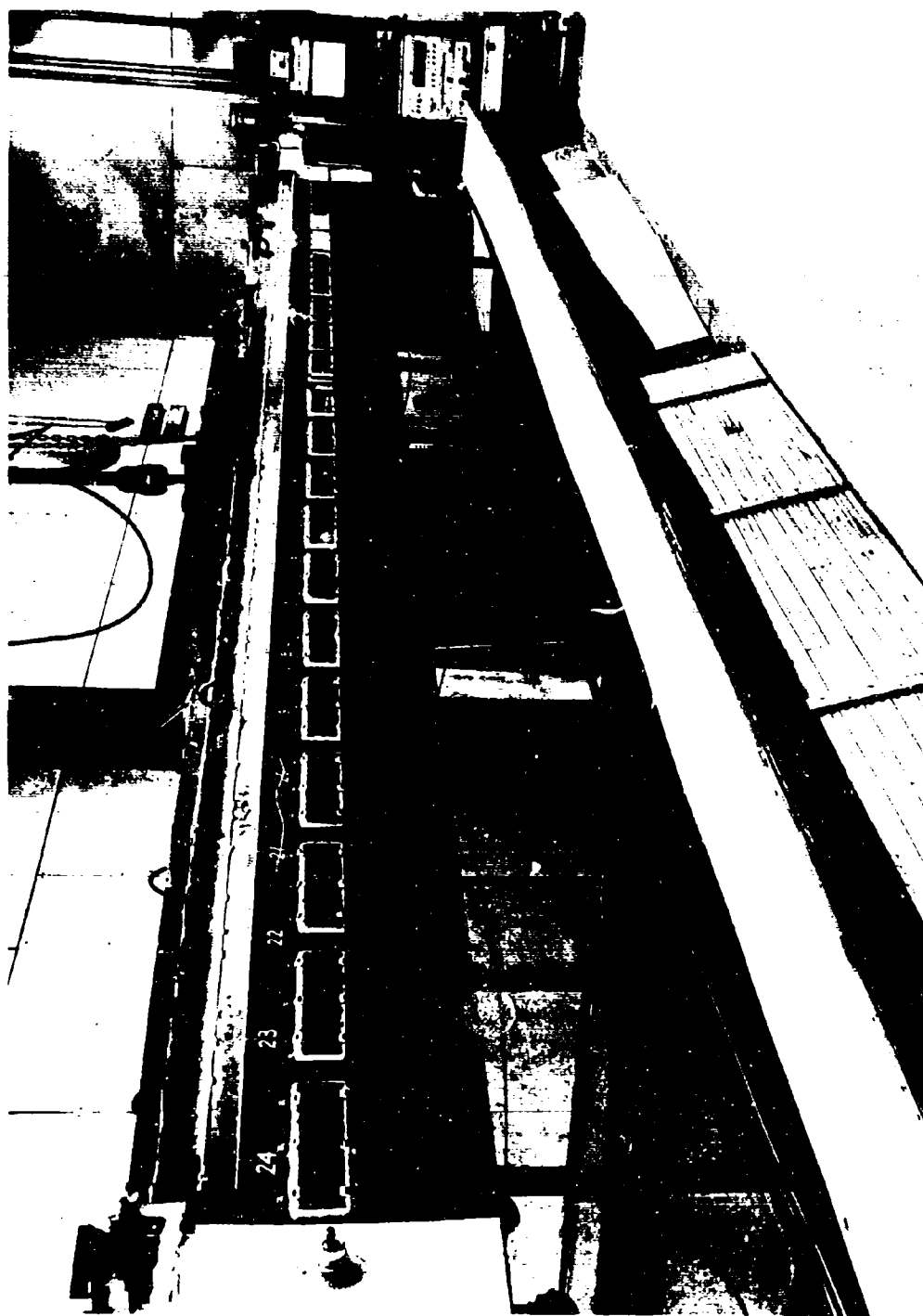


FIGURE 9. ASTME-84 25 FT FLAME SPREAD TUNNEL FURNACE

## 2. Gas Sampling

For analytical purposes, it was necessary to collect a sample of the combustion products in such a manner as to allow their analysis over an extended period of time. This requirement for time was brought about by the need for qualitative and quantitative analysis of a number of different noxious gases.

Two procedures were followed to provide this capability. The first involved the use of a flexible Tedlar<sup>®</sup> bag in which the combustion products sample was collected. The second procedure involved the use of the 10-cu-ft static exposure chamber. Both procedures utilized the E-84 tunnel furnace.

In each case, the combustion product stream in the tunnel furnace was sampled by means of a perforated pipe placed in the center of the furnace cross section at the exhaust end (Figure 10). The perforations were positioned on the upstream side. An air shield displacement pump with a Telfon<sup>®</sup> coated diaphragm was used in line to pump combustion products out of the tunnel furnace (which was under slight vacuum during test) into the Tedlar<sup>®</sup> bag (Figure 11). After one or two tests, however, because of analysis problems, a condensing coil was added to allow removal of the condensables.

In those tests where the 10-cu-ft static exposure chamber was used, the combustion products were pulled into the chamber by means of a vacuum pump placed on the downstream side of the chamber. No condenser was utilized in any of the tests with the exposure chamber.

## 3. Gas Analysis

Analysis of the combustion products was accomplished through the use of three infrared analyzers, two of which monitored the carbon monoxide level (one high scale and one low scale instrument) and one which monitored the carbon dioxide, a paramagnetic oxygen analyzer, and several varieties of the commonly available colorimetric indicator tubes. The low scale carbon monoxide analyzer (0 to 2000 ppm) was an MSA LIRA 300 instrument. Both the high scale carbon monoxide analyzer (0 to 2 percent or 0 to 10 percent) and the carbon dioxide analyzer were Beckman IR 315 s. The oxygen instrument was a Beckman F3.

The colorimetric indicator tubes were of two types as supplied by Unico Environmental Instruments, Inc. (Fall River, Mass.) and the

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<sup>®</sup>DuPont registered trademark.



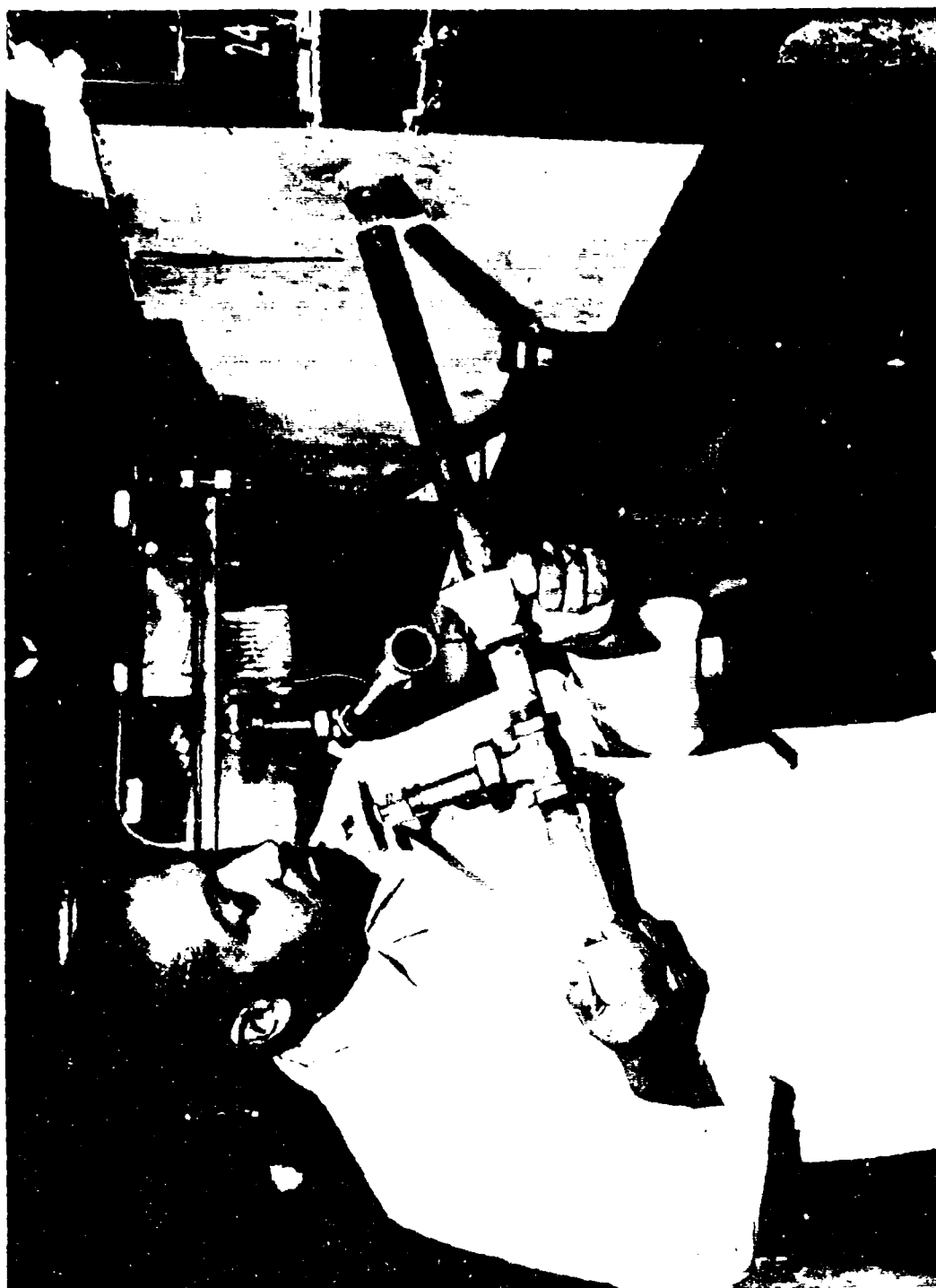


FIGURE 10. COMBUSTION PRODUCT SAMPLE PROBE

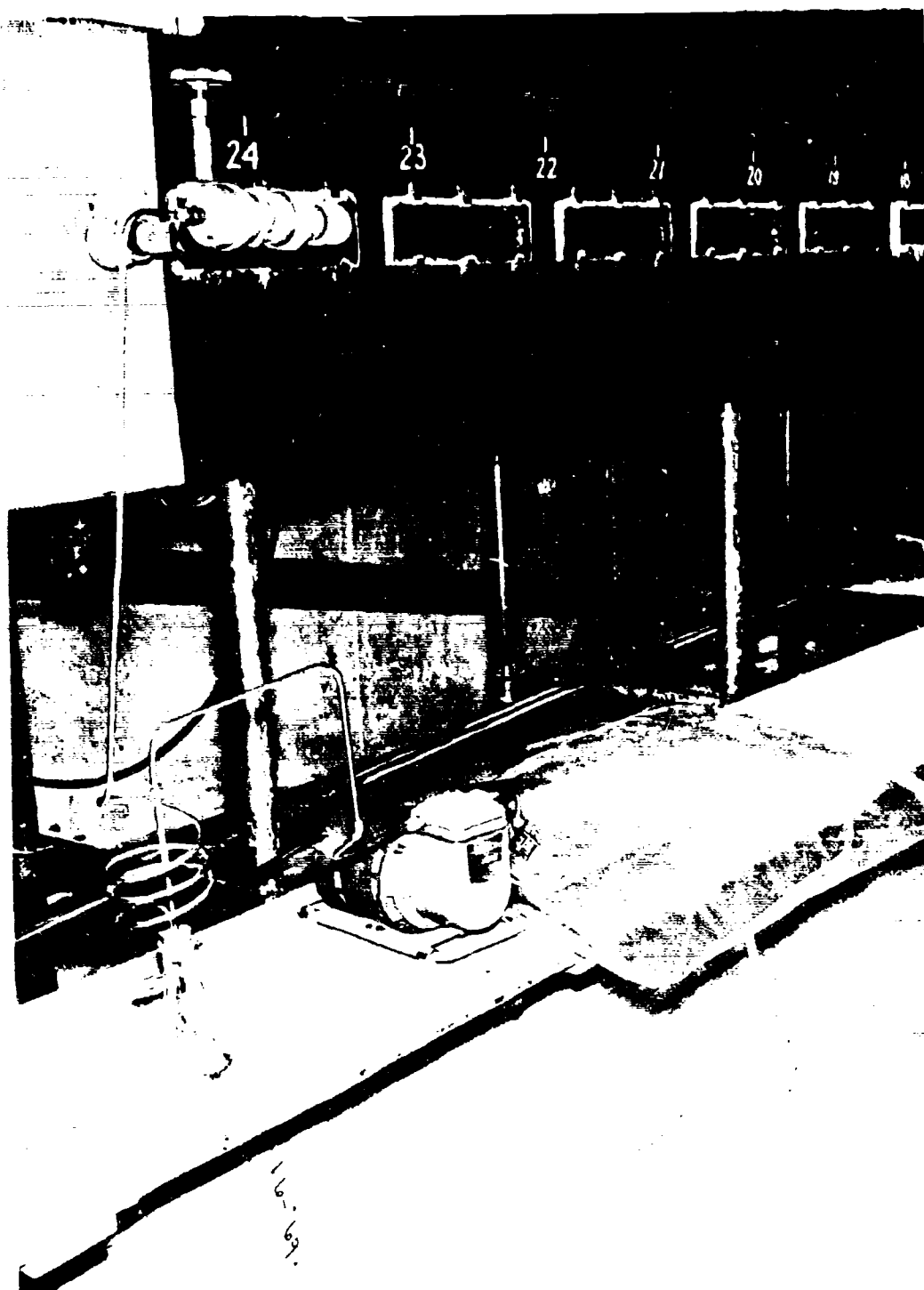


FIGURE 11. COMBUSTION PRODUCT SAMPLE SYSTEM

the Mine Safety Appliances Company (Pittsburgh, Pa). The Unico tubes are frequently referred to as Kitagawa tubes.

In the several experiments where the Tedlar®\* bag was utilized the combustion products were sampled continuously over a 6-min period providing an integrated time-concentration sample. Immediately following this sampling procedure, the bag was then placed in a laboratory hood for analysis of the gases. The colorimetric tubes were then utilized, each in turn and with the appropriate hand pump (Figure 12).

In many cases, fifteen or twenty separate colorimetric tube analyses were completed on a single bag sample. As such, it was not uncommon for the entire analysis procedure to last 45 min or 1 hr. Because of this time lag, a gradual change in the makeup of the sampled gases was suspected. To evaluate this effect, samples were taken at various times as much as 1 hr or more apart and analyzed for particular gases. In all cases, there was little or no change indicated, probably because of the comparatively reduced temperature (70° to 72°F) of the sample.

In those cases where the carbon monoxide or carbon dioxide levels were beyond the range of the available indicator tubes, the infrared analyzers were used to determine the concentrations. The oxygen levels, in all tests where shown, were determined with the Beckman F3. For these determinations, it was a simple matter to attach the Tedlar® bag to the inlet side of the analysis equipment.

Similar procedures were followed in the analysis of combustion products where the static exposure chamber was utilized. The colorimetric tubes were inserted through a small 1/4-in. port in the side of the chamber (Figure 8). Permanent piping between the chamber and the analyzers was provided to allow analysis of carbon monoxide, carbon dioxide, and oxygen levels at any time. The gases so analyzed were vented back into the chamber to minimize the inflow of room air and attendant dilution of the chamber atmosphere.

## C. Definition of Exposure Conditions

### 1. Test Results

A total of fifteen tests were completed in which material was burned and gas analyses conducted to varying degrees. The results are as indicated in Table 3. Where no figure is shown, no analysis was attempted. Where analyses were completed and there was no indication of any measurable quantity, a zero is shown.

\*DuPont registered trademark.



FIGURE 12. SAMPLING FOR ANALYSIS  
WITH COLORIMETRIC TUBES

TABLE 3. ANALYSIS OF COMBUSTION PRODUCTS FROM THE BURNING OF MATERIALS

Test No.	Material	CO	CO <sub>2</sub>	O <sub>2</sub>	HCN	H <sub>2</sub> S	Acetic Acid	SO <sub>2</sub>	NO <sub>2</sub>	Phosgene	Cl	Br	NH <sub>3</sub>	HCl	ClO <sub>2</sub>
1	Acrylic Carpet 4/3	1,100	33,300	150,000	100			0	1		0	0		0	16
2	Wool Carpet 4/3	19,000	180,000	50,000	10,250			0	0		0	0		0	16
3	Wool 4/17	30,000	150,000	100,000	7,500	4		20	10				Trace	Trace	Trace
4	Red Oak 4/17	13,600	93,000	100,000	35	0		50	10				0	0	4
5	D-Fir 5/2	9,400	56,000	110,000	30	0		25	Trace	4	0	0	0	0	0.2
6	Red Oak 5/2	11,000	100,000	100,000	55	10		50	Trace	0.5	0	0	0	0	0
7	D-Fir 5/29 (A)		9,000		1	0	10	5	0	0	0	0	0		
8	D-Fir 5/28				0.5	0	10	4.0	Trace	0					
9	D-Fir 6/5 (A)	1,850	20,000		5	0	0	1.5	0	0	0	0	0	0	
10	D-Fir 6/6		12,000		2	0	10	6	0	0	0	0	0	0	
11	PVC 6/16	1,500												100	
12	Polyester 6/17 (A)	1,025			18	0	0	0	0	0	0	0	0	0	Trace
13	Acrlan 6/18 (A)	1,300	15,000		65	0	0	0	0.3	0	0	5	0	2	0
14	Wool 6/19	1,100			80	0	0	10	0.2	0	0	0	0	0	0
15	Tile 6/20 (A)	900	10,000		10	0	50	15	0	0	0	0	0	0	0

NOTE: These materials were burned in the ASTM E-84 Tunnel Furnace at SwRI under varying conditions.

(A) indicates a test wherein arrivals were exposed to the combustion products.

In Test No. 5 with Douglas-fir, 30 ppm of hydrogen cyanide was recorded. This was not expected and as shown in Tests 7 through 10 was not repeated. The maximum recorded in any later tests was 5 ppm. The high concentration of 30 ppm in Test No. 5 may have been due to the burning of some residue material in the tunnel furnace at the time of test.

The carbon dioxide/carbon monoxide ratios range from about 30 to as low as 5. The high ratios occur at a high oxygen concentration of 15 percent while the low ratios of 5 occur at a low oxygen concentration of 5 percent. These values appear to be within the range expected under "typical" burning conditions and were obtained by varying the ventilation conditions in the tunnel furnace during each test. This was done by changing the airflow through the tunnel furnace through use of the exhaust damper control and the adjustable inlet opening.

## 2. Relative Gas Concentrations and Dilution

In those tests where it was planned to expose the test animals to noxious gases and smoke, it was important to consider the relative makeup of the noxious gases of interest. As completed in this study, these gases were utilized in relative proportion to each other as produced by active burning. The representativeness of the dynamic burning conditions in the tunnel furnace provided this relationship.

In the planning of these exposure tests, it was necessary to insure that the toxicity level of the combined exposure was not so high as to produce deaths in so short a time that little comparative data would be obtained. Thus, after analysis of the combustion products contained within the static exposure chamber was completed, the atmosphere was diluted with room air to provide the toxicity level desired. This method had the advantage of retaining the noxious gases in their relative concentration.

## V. EXPERIMENTAL ANIMAL EXPOSURES

### A. General

In the previous work on OCD Work Unit 2537A, (14) mice were exposed to the four variables of temperature, oxygen, carbon monoxide, and carbon dioxide, individually and in combinations of two and three. Analysis of the data indicated that when the four variables were encountered, as compared to exposures of one at a time, there was a significant and additive increase in toxicity. A further increase was noted when three variables were combined in such a manner. The results of this study clearly indicated the importance of studying the toxicity of variables in combinations rather than studying them individually. This is important since the definition of a toxic environment for occupants of shelters exposed to fire will generally involve combinations of these and other elements.

This investigation showed that more work was required to establish the relative importance of other components of a fire atmosphere, in addition to the four variables studied previously. Some of the components which were suggested as possible contributors to the overall toxicity included hydrogen chloride, nitrogen dioxide, sulfur dioxide, phosgene, and hydrogen cyanide. It was also shown that some additional experiments would be necessary to define the toxicity to carbon monoxide since some erratic results were encountered, perhaps due to chamber design or the methods of monitoring and analysis. It was recommended that, in addition to establishing the carbon monoxide toxicity data and study of the noxious gases, some work be conducted on exposure of the mice to an actual fire atmosphere. This was considered necessary for definition of those additional variables in the combustion products stream that may contribute significantly to toxicity which have as yet been undefined. It was also recommended that a portion of the investigation include the additional variable of time; that is, times longer than the 4 hr previously studied, perhaps up to 24 hr.

### B. Primary Variables

The initial phase of OCD Work Unit 2537B involved an exposure of mice to the variable carbon monoxide with combinations of the other variables (carbon dioxide, oxygen, heat), in order to clearly show toxicity indices. In Work Unit 2537A, there were some inconsistencies in the data whenever carbon monoxide was included. It was postulated that these questionable data were due to inadequate control of carbon monoxide gas mixtures or possibly due to the chamber design itself. During this investigation, improved methods of analysis and monitoring of carbon monoxide levels have been utilized. Also, the use of a larger chamber with individual compartments eliminates the stresses caused by crowding and allows the study of individual variations in terms of other variables such as age and sex of the animals.

A summary of the data found with carbon monoxide in combination with the other three primary variables as developed in the larger chamber used in this study is shown in Table 4. In general, the toxicity to experimental conditions is less in the larger chamber than that shown in the smaller chamber in Work Unit 2537A.<sup>(14)</sup> For example, in the smaller chamber, it took 100°F to produce death in a 4-hr exposure, while, in the larger chamber, it took 110°F to produce death. Exposure of the mice to different concentrations of carbon monoxide in the larger chamber with 21 percent oxygen at 85°F showed that there were no deaths at 0.05 or 0.075 percent carbon monoxide; however, deaths were encountered consistently at the 0.10-percent carbon monoxide level. In the previous study with the smaller chamber, deaths were encountered at levels as low as 0.05 percent. In the smaller chamber there was also a corresponding increase in toxicity at the 0.125 percent level. These results were reproducible within this batch of mice, and no inconsistencies were encountered.

An additional portion of the experimental program was designed to show the effects of combining carbon monoxide with the other primary variables, namely anoxia, heat, and carbon dioxide. The intent was to show whether or not these combinations produced a combined increased toxicity to the variables. This was found with two of the three variables but not with the third, namely carbon dioxide. These results, shown in Table 4, are similar to those seen in Work Unit 2537A.<sup>(14)</sup> The combination of carbon monoxide with anoxia showed that with 16 percent oxygen and 0.075 percent carbon monoxide at 85°F, deaths were encountered consistently. With heat and carbon monoxide, deaths were encountered at 100°F with 0.075 percent carbon monoxide. With carbon dioxide, however, combination of 0.075 percent carbon monoxide with 30 percent carbon dioxide did not produce any deaths of the animals in 4 hr. The concentration of 40 percent carbon dioxide with no added carbon monoxide produced two deaths in 4 hr. Thus, there was no apparent additive effect when carbon dioxide and carbon monoxide were combined in this test at 85°F.

Combinations of three variables, anoxia, carbon monoxide, and heat, showed that deaths were encountered with 16 percent oxygen and 0.05 percent carbon monoxide at a temperature of 100°F. Also, deaths were encountered with 18.5 percent oxygen and 0.075 percent carbon monoxide at a temperature of 95°F. Combinations of anoxia, carbon monoxide, and carbon dioxide did not show any additive effect, with the possible exception that the combination of 13.5 percent oxygen, 0.05 percent carbon monoxide, and 30 percent carbon dioxide at a temperature of 85°F produced one death, whereas the same test without the carbon dioxide produced three deaths. This may not be significant; however, the animal tests were run at the same time. From these results, it is apparent that an increased toxicity resulted with the elimination of carbon dioxide, which indicates an antagonistic effect when combined with anoxia, carbon monoxide, and 85°F.



TABLE 4. RESULTS OF ANIMAL EXPOSURES TO FIRE GASES

	Percent by Volume			Temperature, °F	Survivors Out of 10
	O <sub>2</sub>	CO	CO <sub>2</sub>		
Carbon Monoxide	21	0.125	-	85	6
	21	0.10	-	85	8
	21	0.10	-	85	9
	21	0.075	-	85	10
	21	0.05	-	85	10
Heat	21	-	-	95	10
	21	-	-	100	10
	21	-	-	105	10
	21	-	-	110	8
	21	-	-	115	6
Anoxia + Carbon Monoxide	11	0.075	-	85	6
	13.5	0.075	-	85	5
	16	0.075	-	85	8
	16	0.075	-	85	9
	18.5	0.075	-	85	10
Heat + Carbon Monoxide	21	0.05	-	95	10
	21	0.075	-	95	10
	21	0.075	-	100	8
Carbon Dioxide +	21	0.075	20	85	10
	21	0.075	25	85	10
Carbon Monoxide	21	0.075	30	85	10
	21	-	40	85	8
Carbon Monoxide + Anoxia + Heat	16	0.05	-	100	8
	18.5	0.05	-	95	10
	18.5	0.05	-	100	10
	18.5	0.075	-	95	9
Carbon Dioxide + Anoxia +	18.5	0.05	20	85	10
	18.5	0.05	30	85	10
	18.5	0.075	30	85	10
	16.0	0.075	30	85	7
Carbon Monoxide	13.5	0.05	-	85	7
	13.5	0.05	30	85	9
Carbon Dioxide + Heat +	21	0.05	30	95	10
	21	0.075	30	95	9
	18.5	0.05	20	95	10
	18.5	0.05	30	95	8
Carbon Monoxide + Anoxia					

A combination of carbon dioxide with heat and carbon monoxide produced a different result. Addition of 30 percent carbon dioxide to 21 percent oxygen and 0.075 percent carbon monoxide at 95°F produced one death, whereas in an earlier experiment without the carbon dioxide there were no deaths. Also, the test with 18.5 percent oxygen and 0.05 percent carbon monoxide with 30 percent carbon dioxide at 95°F produced two deaths. This same condition without the carbon dioxide did not produce death, which indicates that the carbon dioxide is contributing to the toxicity.

A latter part of this investigation included determination of the effects of exposures to these four variables for periods longer than 4 hr. These tests were carried out in the flow-through chamber. As used in this portion of the study, it included the compartments which were utilized to determine individual activity variations. Table 5 includes a summary of the data found in exposing the four primary variables, carbon monoxide, heat, carbon dioxide, and anoxia, for periods up to 24 hr.

Prior to the beginning of this phase of the work, a new batch of mice was received from the supplier and it was determined that the general response of these mice to all of the variables studied was different from that of mice used previously. These mice were the same strain and from the same supplier; however, for some unknown reason, they did give a slightly different response to the variables. In general, these mice were not as sensitive to the different exposure levels. This can be misleading if results are not made on a comparative basis within a batch of mice. Thus, the data reported in this table should be considered by themselves and not compared directly to the previous data. Further work would be necessary to fully define the importance of age and other differences; however it is not of primary importance to this study at this time.

Examination of the data indicates there was an apparent increase in toxicity with the variable carbon monoxide when exposure times were extended to 24 hr. After a 4-hr exposure at 0.15 percent carbon monoxide, there were no deaths. When the time was extended to 24 hr at 0.15 percent carbon monoxide, five deaths were found. Extension of the exposure period beyond 4 hr and up to 24 hr did not affect the toxicity with respect to heat, carbon dioxide, and anoxia (Table 5). All deaths encountered with these three variables were seen within the 4-hr exposure period and continued exposure periods up to 24 hr did not produce any additional deaths.

### C. Noxious Gases

Four additional noxious gases were evaluated for their toxicity to mice. These variables were evaluated individually and in combination with the previously-studied primary variables. Hydrogen chloride, nitrogen dioxide, sulfur dioxide, and hydrogen cyanide were selected as the four noxious gases. It was discovered that the metering of the very small

TABLE 5. RESULTS OF ANIMAL EXPOSURE TO  
THE PRIMARY VARIABLES

	Percent by Volume			Temp (°F)	Time (hr)	Survivors (out of 10)
	O <sub>2</sub>	CO	CO <sub>2</sub>			
Carbon Monoxide	21	0.10	--	85	24	10
	21	0.125	--	85	24	10
	21	0.150	--	85	24	5
	21	0.150	--	85	4	10
	21	0.175	--	85	4	8
Heat	21	--	--	100	24	10
	21	--	--	110	24	10
	21	--	--	115	24	10
	21	--	--	120	24	7
	21	--	--	120	4	7
Carbon Dioxide	21	--	40	85	24	10
	21	--	50	85	24	8
	21	--	50	85	4	8
Anoxia	16	--	--	85	24	10
	11	--	--	85	24	10
	9	--	--	85	24	10
	7.5	--	--	85	24	6
	7.5	--	--	85	4	7

quantities of the individual noxious gases into the chamber produced mechanical difficulties not encountered previously. Since these gases are polar, they readily absorb into surfaces. It was determined that much if not all of these gases were absorbed and lost in the flow-through animal chamber. Removal of the individual compartment apparatus from the chamber resulted in a satisfactory metering of sulfur dioxide, nitrogen dioxide, and hydrogen cyanide; however, hydrogen chloride was still absorbed, apparently quite well. Attempts to alleviate this condition with hydrogen chloride were not successful; thus, this gas was not studied in this series of animal tests.

A study of the effects of nitrogen dioxide on the toxicity of mice showed that deaths were encountered in a 4-hr period with a concentration of 100 parts per million (ppm). With 200 ppm of nitrogen dioxide, there were no survivors within the 4-hr exposure period. With sulfur dioxide, deaths were encountered when the concentration reached 800 ppm. A similar study with hydrogen cyanide showed that deaths were encountered with 100 ppm of cyanide. A summary of the test data from these gases is shown in Table 6.

Exposure of these noxious gases for periods longer than 4 hr indicated that there was significant number of animals that died between the 4- and the 24-hr exposure with hydrogen cyanide, but none were similarly affected with sulfur dioxide and nitrogen dioxide.

#### D. Primary Variables Plus Noxious Gases

The noxious gases (sulfur dioxide, hydrogen cyanide, and nitrogen dioxide) were combined with the previously-studied primary variables (heat, carbon monoxide, carbon dioxide, and anoxia) to provide an indication of the degree of any synergistic or additive effects. A summary of results is shown in Table 7.

Examination of the data indicates a rather dramatic synergistic effect with each of the three noxious gases. For example, with nitrogen dioxide as little as 10 ppm, added to 16 percent oxygen, 0.075 percent carbon monoxide, 30 percent carbon dioxide, and 100°F, produced two deaths within 4 hr. Exposure of animals up to 24 hr with these same conditions minus the 10 ppm of nitrogen dioxide did not produce any deaths. Also, it should be noted that with nitrogen dioxide alone, it required 100 ppm to produce deaths.

A similar relationship was found with combinations of sulfur dioxide and hydrogen cyanide with the four primary variables. Thus, with 25 ppm of sulfur dioxide, there were three deaths, and, with 10 ppm with hydrogen cyanide, there were two deaths. For all three of the gases studied, these are considered to be significant synergistic effects. If only additive effects had been indicated, then roughly 75 ppm of nitrogen dioxide would have been expected to produce one to three deaths in this type of combination; with

TABLE 6. RESULTS OF ANIMAL EXPOSURES TO NOXIOUS GASES

	Percent by Volume			ppm		Temp (°F)	Time (hr)	Survivors (out of 10)
	O <sub>2</sub>	CO	CO <sub>2</sub>	NO <sub>2</sub>	SO <sub>2</sub>			
Nitrogen Dioxide	21	--	--	50	--	85	4	10
	21	--	--	100	--	85	4	7
	21	--	--	200	--	85	4	0
Sulfur Dioxide	21	--	--	--	250	85	4	10
	21	--	--	--	300	85	4	10
	21	--	--	--	400	85	4	10
	21	--	--	--	600	85	4	10
	21	--	--	--	800	85	4	7
Hydrogen Cyanide	21	--	--	--	--	85	4	9
	21	--	--	--	--	85	4	0
	21	--	--	--	--	85	12	0
	21	--	--	--	--	85	24	10

TABLE 7. RESULTS OF COMBINED EXPOSURES,  
PRIMARY VARIABLES PLUS NOXIOUS GASES

	Percent by Volume			ppm			Temp (°F)	Time (hr)	Survivors (out of 10)
	O <sub>2</sub>	CO	CO <sub>2</sub>	NO <sub>2</sub>	SO <sub>2</sub>	HCN			
CO <sub>2</sub> + CO + Heat + Anoxia + NO <sub>2</sub>	16	0.075	30	5	-	-	100	4	10
	16	0.075	30	10	-	-	100	4	8
CO <sub>2</sub> + CO + Heat + Anoxia + SO <sub>2</sub>	16	0.075	30	-	15	-	100	4	10
	16	0.075	30	-	25	-	100	4	7
	16	0.075	30	-	80	-	100	4	0
	16	0.075	30	-	-	-	100	4	0
CO <sub>2</sub> + CO + Heat + Anoxia + HCN	16	0.075	30	-	-	5	100	4	10
	16	0.075	30	-	-	10	100	4	8
	16	0.075	30	-	-	30	100	4	0
	16	0.075	30	-	-	75	100	4	0
CO <sub>2</sub> + CO + Heat + Anoxia	16	0.075	30	-	-	-	100	4	10
	16	0.075	30	-	-	-	100	24	10

sulfur dioxide, it would have been about 700 ppm; and with hydrogen cyanide, 75 ppm. Obviously, the presence of these very small amounts of noxious gases produces a considerable increase in toxicity with respect to the other variables.

#### E. Combustion Products

The exposure of animals to combustion products, including smoke, was carried out in the static exposure chamber as described previously. Most of the exposures were accomplished using the combustion products from the burning of Douglas-fir in the ASTM E-84 flame spread tunnel furnace. Tables 8 through 18 contain the test conditions and results. In each instance, except that reported in Table 18, ten mice (five male, five female) were placed in the chamber at time zero. The results of these tests should not be used as a measure of their relative hazard because of the variations imposed to produce combustion products within the desired ranges.

The first test run in this series, described in Table 8, was initially set up for 900 ppm of carbon monoxide at 85°F. However, after 3 hr, the level had dropped to 600 ppm and no deaths were recorded. Modifications of the chamber were then completed to provide a more airtight closure.

In the second test, see Table 9, somewhat better control was established using the combustion products from the burning of Douglas-fir. Initial conditions were set at 100°F, with 1100 ppm of carbon monoxide. Over the 4-hr test period, one death was recorded while the carbon monoxide level dropped to 900 ppm.

In a third test with Douglas-fir combustion products (see Table 10), initial conditions were set at 100°F, with 1560 ppm of carbon monoxide. During a 4-hr exposure, two animals died while the carbon monoxide level dropped to 1050 ppm. For control purposes, this nearly one-third reduction was considered excessive.

After modification of the exposure chamber to allow adjustment of the carbon monoxide level, a fourth test was completed with Douglas-fir combustion products (see Table 11). Initial conditions were set at 100°F, with a carbon monoxide level of 1600 ppm. During a 4-hr exposure with a carbon monoxide level maintained between 1600 and 1400 ppm, seven animals died.

Following the previous test, a control experiment was conducted to evaluate the effects of the noxious gases encountered with Douglas-fir. In this test (see Table 12), the chamber was conditioned to 100°F with a 1500-ppm carbon monoxide level. During this 4-hr exposure, two animals died. These results compare with the previous test (see Table 11) in which seven animals died, thereby suggesting a significant effect of the smoke and/or noxious gases.

TABLE 8. EXPOSURE TEST WITH COMBUSTION PRODUCTS  
FROM THE BURNING OF DOUGLAS-FIR

Chamber at 85°F, CO = 600 to 900 ppm  
Analysis of gases contained in Table 3, Test No. 7

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00 to 0:15	Squinting, restlessness, excessive grooming of nose, intermittent, agitated spurts, and gasping (CO = 900 ppm)
1:00	All animals quiet, shallow breathing (CO = 750 ppm)
2:00	Nine animals quiet, one active (CO = 690 ppm)
3:00	Eight animals quiet, two appear irritable (CO = 600 ppm)
4:00	Ten survivors

TABLE 9. EXPOSURE TEST WITH COMBUSTION PRODUCTS  
FROM THE BURNING OF DOUGLAS-FIR

Chamber at 100°F, CO = 900 to 1100 ppm  
Analysis of gases contained in Table 3, Test No. 9

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals quiet and squinting (CO = 1100 ppm)
1:00	Animals quiet, four perspiring heavily
2:00	Animals appear near exhaustion
2:40	One animal dead (male)
3:00	Animals quiet, shallow and quick respiration
4:00	No additional deaths, nine survivors (CO = 900 ppm)



TABLE 10. EXPOSURE TEST WITH COMBUSTION PRODUCTS  
FROM THE BURNING OF DOUGLAS-FIR\*

Chamber at 100°F, CO = 1050 to 1560 ppm

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	Animals curiously active, excessive grooming (CO = 1560 ppm)
0:15	Two mice show agitated spurts
1:00	All animals quiet (CO = 1350 ppm)
2:00	No change in animals
2:10	One animal dead (female) (CO = 1250 ppm)
3:00	Animals quiet, noticeable sweating
3:22	Second animal dead (male) (CO = 1100 ppm)
4:00	Animals quiet, eight survivors (CO = 1050 ppm)

\*The combustion products obtained from the burning of Douglas-fir were not analyzed beyond the determinations of carbon monoxide as indicated.

TABLE 11. EXPOSURE TEST WITH COMBUSTION PRODUCTS  
FROM THE BURNING OF DOUGLAS-FIR

Chamber at 100°F, CO = 1400 to 1600 ppm

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals moderately curious (CO = 1600 ppm)
0:10	All animals squinting, respiration irregular
0:15	Activity erratic, eyes watering
0:18	Four animals hyperactive
0:30	(CO = 1450 ppm)
0:40	Chamber recharged (CO = 1600 ppm)
1:00	Intermittent activity, no deaths
1:30	Animal No. 3 showing agitated spurts, others inter- mittently active
1:43	Animal No. 3 (male) dead, CO = 1500 ppm, other animals appear exhausted
2:18	Second animal dead (male)
2:23	Third animal dead (male)
2:36	Fourth animal dead (female)
2:57	Fifth animal dead (male) (CO = 1400 ppm)
3:05	Sixth animal dead (female)
3:15	(Chamber recharged to 1550 ppm)
3:33	Seventh animal dead (female)
4:00	Three survivors

TABLE 12. EXPOSURE TEST WITH CARBON MONOXIDE  
AND ELEVATED TEMPERATURE

Chamber at 100°F, CO = 1500 ppm

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals curiously active (CO = 1500 ppm)
0:15	Excessive grooming, irritability, increased respiration
0:45	Animals showing agitated spurts
0:48	One animal dead (male), others showing occasional movement
1:30	Second animal dead (male)
2:00	All animals show ataxia
3:00	No change
4:00	No change, eight survivors

TABLE 13. EXPOSURE TEST WITH CARBON MONOXIDE  
AND ELEVATED TEMPERATURE

Chamber at 100°F, CO = 1100 ppm

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals curiously active
1:00	Animals show excessive grooming
2:00	Increased respiration
3:00	No change
4:00	Test terminated, no deaths

TABLE 14. EXPOSURE TEST WITH COMBUSTION PRODUCTS  
FROM THE BURNING OF POLYVINYL CHLORIDE

Chamber at 100°F, CO = 1500 ppm

The analyses of gases utilized in this test were, as contained in Table 3, Test No. 11, diluted with normal air to provide the precise CO level desired.

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals curiously active (CO = 1500 ppm, HCl = 100 ppm)
0:05	Animals squinting, agitated spurts, watery eyes
0:10	Increased respiration, quiet with occasional movement
0:20	Animal No. 4 hyperactive
0:55	Animal No. 4 dead (male), others intermittently active
1:00	(CO = 1500 ppm, HCl = 2 ppm)
1:14	Second (male) and third (female) animals dead
1:30	Fourth (male), fifth (male), and sixth (female) animals dead
2:08	Seventh animal dead (female)
2:30	Eighth animal dead (female)
2:45	Ninth animal dead (female)
3:00	Test terminated, one survivor; this animal (male) died 2 days later

TABLE 15. EXPOSURE TEST WITH COMBUSTION PRODUCTS FROM  
THE BURNING OF A POLYESTER FIBER-GLASS COATING

Chamber at 100°F, CO = 1025 ppm

The analyses of gases utilized in this test were, as contained in Table 3,  
Test No. 12, diluted with normal air to provide the precise CO level desired.

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals curiously active (CO = 1025 ppm)
0:05	Animals squinting, eyes watering
0:15	All animals hyperactive
0:30	Intermittent agitated spurts from all animals
1:00	All animals sweating and hyperactive
2:00	No change
3:00	No change
3:48	One dead (male)
4:00	Nine survivors

TABLE 16. EXPOSURE TEST WITH COMBUSTION PRODUCTS  
FROM THE BURNING OF ACRYLIC CARPETING

Chamber at 100°F, CO = 1300 ppm

The analyses of gases utilized in this test were, as contained in Table 3, Test No. 13, diluted with normal air to provide the precise CO level desired.

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals curiously active
0:05	Quiet occasional movement, squinting
0:15	Animals show intermittent agitated spurts and increased respiration
0:45	Animal No. 8 having convulsive seizure, other animals quiet
0:55	Animal No. 8 dead (female)
1:00	Animal No. 1 having agitated spurts
1:15	Animal No. 1 dead (male)
1:40	Third animal (male) dead following convulsions
1:45	Fourth animal (male) dead in similar fashion
2:00	Survivors quiet
2:15	Fifth animal dead (female)
2:22	Sixth animal dead (female)
3:00	Seventh (female) and eighth (male) animals dead, two survivors, one male and one female

TABLE 17. EXPOSURE TEST WITH COMBUSTION PRODUCTS  
FROM THE BURNING OF WOOL CARPETING

Chamber at 100°F, CO = 1100 ppm

The analyses of gases utilized in this test were, as contained in Table 3, Test No. 14, diluted with normal air to provide the precise CO level desired.

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals curiously active
0:05	Animals squinting, grooming
1:00	Animals quiet, sweating
2:00	No change
3:00	No change
4:00	No change, ten survivors

TABLE 18. EXPOSURE TEST WITH COMBUSTION PRODUCTS  
FROM THE BURNING OF VINYL-ASBESTOS FLOOR TILE

Chamber at 85°F, CO = 800 to 900 ppm

The analyses of gases utilized in this test were, as contained in Table 3, Test No. 15, diluted with normal air to provide the precise level of CO desired.

<u>Time (hr:min)</u>	<u>Animals' Condition</u>
0:00	All animals curiously active (CO = 900 ppm)
0:05	Gasping and grooming
0:20	Animals generally active
0:60	All animals quiet and sweating
2:00	All animals quiet
3:00	No change
4:00	Three females dead, seventeen survivors (CO = 800 ppm)

A second comparative control test was then completed (see Table 13) at a level of 100°F, with 1100 ppm of carbon monoxide. During a 4-hr exposure, no deaths were recorded.

The next five tests were then completed using materials other than Douglas-fir. In the first of this series, a polyvinyl chloride was burned and the combustion products directed into the exposure chamber (see Table 14). The gases were diluted to provide 1500 ppm of carbon monoxide at 100°F. Analysis also indicated 100 ppm of hydrogen chloride at the beginning of the test. After 3 hr, nine animals were dead (the surviving animal died during the post-test observation period). The measurable hydrogen chloride had dropped to 2 ppm after 1 hr.

The following test was completed with the combustion products by burning a polyester fiber-glass coating (see Table 15). The chamber was conditioned at 100°F and the carbon monoxide level maintained at 1025 ppm. Within 4 hr, one animal died.

The third test in this series was completed using a piece of acrylic carpeting (see Table 16). Again, the chamber was conditioned to 100°F while the carbon monoxide level was maintained at 1300 ppm. Within 3 hr, eight of the animals died.

The fourth test was conducted with the combustion products from a piece of wool carpeting. The chamber was set at 100°F with the carbon monoxide level at 1100 ppm (see Table 17). After 4 hr, no deaths were recorded.

The last test in this series was completed using vinyl asbestos floor tile to provide the combustion products (see Table 18). A total of twenty animals were placed in the chamber after conditioning at 100°F with 900 ppm of carbon monoxide. By the end of a 4-hr exposure, three animals had died.

## F. Discussion of All Test Results

### 1. General

Exposure of animals to the primary variables, singly and in combinations, produced additive effects with the exception of the effects of carbon dioxide. Carbon dioxide was additive in some cases and antagonistic in others. Combination of the primary variables (carbon monoxide, carbon dioxide, hypoxia, and hyperthermia) with the noxious gases studied produced synergistic effects when any one of the three gases (nitrogen dioxide, hydrogen cyanide, or sulfur dioxide) was added to the four primary variables. Much greater toxic effects were produced than those that should have been obtained if only additive effects were seen. These results suggest that the addition



of small quantities of these gases produce increased toxicity over the primary combinations.

## 2. Static Exposure Tests

Further study of the combustion products from the actual burning of materials, including smoke and other noxious gases, indicated increased toxicity. Thus, with smoke and other gases from Douglas-fir and 1500 ppm of carbon monoxide with 100°F temperature produced seven deaths, but the corresponding control study with 1500 ppm of carbon monoxide at 100°F without smoke and other gases produced only two deaths. Also, 1100 ppm of carbon monoxide with the combustion products produced three deaths, and, in the control under similar conditions without combustion products, produced no deaths. Table 19 allows a comparison of these relative effects. Study of additional products indicated that hydrogen chloride, which was generated in sufficient quantity in combination with other gases and smoke, may be extremely toxic and should be studied in detail. The burning of materials suggests that there are additional toxic elements in combustion atmospheres other than the primary variables (carbon monoxide, carbon dioxide, oxygen, and heat), which have been studied previously.

## 3. Physiological Responses

In addition to these activity measurements, some general observations of physiological responses were noted in all studies, including the exposure to combustion products. Exposure of mice to conditions of the single variable, anoxia, often showed dyspnea (deep breathing), restlessness, and intermittent convulsive seizures preceding death of some of the animals. With increased temperature, there was profuse sweating and copious salivation preceding what were apparently rather exhausting deaths. Exposure to carbon monoxide was accompanied by increased respiration and ataxia (staggering movements). With exposure to carbon dioxide, there was deep, labored respiration. Exposure to nitrogen dioxide produced shallow, quick, and frequently sporadic respiration (apnea). Exposure to sulfur dioxide produced gasping and, in some animals, a high state of irritability just before death. Exposure to hydrogen cyanide produced no observable physiological unusual effects. Exposure to combinations of these variables eliminated most of the individual observations seen with the single variables; however, in most tests, there was dyspnea (deep breathing), erratic activity, and agitated spurts just prior to death. Occasional sweating was frequently observed. Exposure of the animals to the smoke atmosphere produced squinting, watering of the eyes, and profuse sweating.

## 4. Post-Test Observations

In all of the exposures of mice to different variables, the survivors were observed for 10 days in order to insure the recording of any

TABLE 19. COMPARATIVE TOXICITY OF THE STATIC  
EXPOSURE TESTS (TABLES 8 TO 17)

<u>Test Condition</u>	<u>CO Level, ppm</u>	<u>No. of Deaths (ten animals per test)</u>
Douglas-fir	900-1100	1
Douglas-fir	1050-1560	2
Douglas-fir	1400-1600	7
Control	1500	2
Control	1100	0
PVC	1500	10
Polyester	1025	1
Acrylic	1300	8
Wool	1100	0
Vinyl/Asbestos	800-900	3*

\*Twenty animals were utilized in this test.

post-exposure deaths. With sulfur dioxide and nitrogen dioxide, deaths were seen 24 to 48 hr following exposure. No other post-exposure deaths were observed.

5. Sex As a Variable

In each exposure test, five female and five male mice were used. Deaths were fairly evenly divided, with male deaths consistently higher than female. Further data should provide additional understanding of this problem.

## VI. ACTIVITY STUDIES

### A. General

As a part of this program, observations were made to determine whether or not the physical activity of the animals in the various tests influenced the toxicity of the individual mice. It has been shown that there is an individual variability in response to any of the toxic environments studied thus far; for example, in some of the exposures, one to two mice have died early in the 4-hr exposure period while the other mice have not died even when exposures were continued up to as long as 24 hr. The question may be asked whether or not these particular mice had an increased physical activity which influenced their toxicity. In order to answer this question, the physical activity of the mice in the exposures was recorded.

### B. Procedures

The procedures used for determining the activity of the animals were described in Section III. B. 2.

### C. Results

The results indicate that there is no clear-cut correlation between activity and toxicity with respect to individual mice. Table 20 is a summary of the activity measurements. It shows that the activity level for survivors was very similar to that of animals which died and that the average activity did not change appreciably during the test period. Although not shown in this table, activity measurements were slightly higher for the first 15 to 30 min that the animals were in the chamber and then decreased to a rating of between two and three for the remainder of the test period. This may be said, in general, for those mice which survived the exposures. For those mice which did not survive, an erratic increase in activity was frequently seen immediately preceding death. This activity may be described as convulsions in many cases and, in general, may be a result of the injury level at that time rather than the cause. Nevertheless, an overall observation suggests that regardless of the type of exposure and whether or not the animals died, they were relatively inactive.

### D. Discussion

Although the results indicate that activity did not influence the results seen in this study, it does not mean that activity is not an important factor to consider in evaluating toxicity in a mass fire environment. These results merely indicate that the mice in these exposure chambers were relatively inactive throughout the exposure period. In order to evaluate activity as an additional variable in a fire environment, it is necessary to introduce some sort of physical activity and then determine toxicity to the various elements as compared with some measurable level of activity.

TABLE 20. SUMMARY OF PHYSICAL ACTIVITY DATA

Time Period (X) (min)	Average Activity Score for Animals Dying in Time Period (X)	Average Activity Score for Survivors at Time Period (X)	Average Activity Score for All Nonsurvivors at Time Period (X)	Average Activity Score for All Survivors at Time Period (X)
75-80	2.9	2.6	2.9	2.7
80-85	2.4	2.4	2.4	2.7
85-90	2.3	2.4		
	3.0	2.3	3.2	2.6
	4.2	3.1		
95-100	2.8	3.3	2.8	2.6
100-105	2.4	2.3	2.4	2.6
105-110	3.3	2.3	3.3	2.5
110-115	2.7	3.1		
	2.5	2.3	2.7	2.5
	3.3	2.9		
115-120	3.0	2.5		
	3.8	2.9	3.4	2.5
130-135	3.1	2.6		
	2.8	2.4	2.9	2.5
140-145	3.2	2.5		
	2.5	2.3	2.5	2.4
145-150	2.6	2.4		
	2.6	2.4	2.6	2.4
	2.7	2.4		
150-155	3.3	2.3		
	2.5	2.4	2.6	2.4
155-160	2.6	2.5	2.6	2.4
165-170	2.6	2.2	2.6	2.4
170-175	2.6	2.3		
	2.7	2.4	2.6	2.4
175-180	2.6	2.4	2.6	2.4
185-190	2.4	2.5		
	2.1	2.2		
	2.5	2.3	2.5	2.4
	3.1	2.8		
190-195	2.5	2.3		
195-200	2.7	2.2	2.7	2.4
205-210	2.3	2.4		
	2.6	2.3	2.4	2.4
	2.3	2.2		
210-215	2.4	2.2	2.4	2.3
215-220	2.3	2.3		
	2.5	2.3		
	3.1	2.4	2.7	2.3
	2.5	2.2		
220-225	2.7	2.2		
	2.8	2.4	2.7	2.3
	2.6	2.3		
225-230	2.3	2.1	2.3	2.3
230-235	2.3	2.3		
	2.4	2.6	3.7	2.3
	4.3	2.7		
230-240	2.6	2.4		
	2.3	2.3		
	2.2	2.4	2.5	2.3
	2.8	2.5		
	2.2	2.2		
	3.1	2.9		

## VII. PATHOLOGICAL STUDIES

### A. General

At the completion of each test, all surviving animals were placed in observation cages for a 10-day post-test period. Those animals that died during each exposure were necropsied and grossly examined. Tissues were saved (trachea, lungs, heart, kidneys, liver, and spleen) from those identified as the first and last to die in each test. These tissues were initially preserved in formalin and later blocked in paraffin for storage and later sectioning.

At the end of the 10-day post-test observation period, the surviving animals were euthanized, and approximately 50 percent of these were necropsied and grossly examined. No tissues were saved from this group.

### B. Gross Examinations

The necropsies and gross examinations performed on all animals that died during the 4-hr exposures revealed no lesions, but generalized congestion was present. There was no indication that there was any injury or irritation of the pharynx or upper respiratory tree.

Gross examination of approximately half the animals that survived the 4-hr exposures and the 10-day post-test observation period did not reveal any observable lesions or congestion.

### C. Histopathological Analysis

Microscopic slides of the tissues were prepared from a cross section of the different exposures. These slides were cut [stained with hemotoxylin and eosin (H&E)] from those blocks which contained the lungs and trachea. Tissues from 76 animals were included in this histopathological study.

The same conditions were revealed in these examinations as were indicated in the gross examinations conducted immediately after each exposure. The only sign of trauma evident was congestion of the lungs and vascular system.

### D. Discussion

The degree of congestion of the lungs and vascular system was approximately the same in each animal and each group. There was little evidence of any differences as far as gross lesions were concerned between the groups treated with different materials. The microscopic examination of the trachea and lungs showed no change in the tissues other than congestion.

There is a possibility that micro-changes could have occurred, but, to observe such changes, electron microscopy would be required.

Congestion and the absence of lesions as revealed in gross examinations and histopathological studies are the typical findings when death is due to heat prostration and/or heat and anoxia. (34)

In some animals, it was observed that single variables influenced activity and produced characteristic signs of excitation and irritability prior to death. However, when each of the noxious gases, nitrogen dioxide, sulfur dioxide, and hydrogen cyanide, was combined with a fixed atmosphere of anoxia, temperature, carbon monoxide, and carbon dioxide, their individual signs of excitation and irritability were undistinguishable. Dyspnea, erratic activity, agitated spurts, and convulsions preceded all deaths. The gross necropsy examinations and histopathological analysis showed that the varied activity and signs of toxicity preceding death were not reflected by varied alterations at the tissue level.

### VIII. DISCUSSION AND CONCLUSIONS

The series of animal tests completed during the past year was designed to determine the effects of various combinations of fire gases likely to be present in a mass fire atmosphere. The purpose was to evaluate the relative contributions of these various components encountered in realistic combinations. The emphasis was to extend the study beyond the four previously studied primary variables--temperature, oxygen, carbon monoxide, and carbon dioxide--and to evaluate combinations with other noxious gases such as hydrogen cyanide, sulfur dioxide, hydrogen chloride, and nitrogen dioxide. The program was also designed to evaluate these gases produced by actual burning situations to establish what elements contribute to the toxicity in addition to those already studied.

All of the test data indicate that combinations of variables produced at least additive effects. This is important to consider in prescribing toxicity limits of various elements likely to be encountered in a fire situation. When noxious gases such as hydrogen cyanide, sulfur dioxide, and nitrogen dioxide were combined with the previously studied primary variables, however, synergistic effects were noted. Thus addition of a very small quantity (as little as 10 ppm) of one of these noxious gases to the four primary variables produced a toxic situation not seen in the absence of the newly added gas. The noxious gas alone produced toxicity at 10 times this level.

Hydrogen chloride gas was originally scheduled to be studied during this test, however, the design of the chamber was such that the small concentrations used were rapidly absorbed by the chamber material. It is likely that this gas will be an important one for consideration in the fire atmosphere and should be studied because of its toxic nature toward lung tissue. Effects of other gases--ammonia, chlorine, hydrogen sulfide, nitric oxide, and many others--also have not been established.

Additional exposure tests were conducted to evaluate the toxicity of a complete smoke atmosphere on mice to allow comparison of these results with those obtained with the artificial atmospheres containing the primary variables plus specified noxious gases. The purpose of completing these tests was to show whether or not there were sufficient quantities of unknown gases or other materials present in a fire atmosphere which contributed to the total toxic effect and to define the degree of these effects. Results indicate that there are unidentified components in combustion gases which contribute to the total toxicity. The investigation showed clearly that improved methods of analysis, i. e., beyond those utilized in this program, of the various components of the smoke atmosphere are needed to characterize these components. Exposure of animals to the combustion products will also require a system of burning of various materials specifically designed for this purpose.



Part of the overall animal exposure was an attempt to relate the physical activity of animals with various factors such as the type of exposure and the resultant toxicity to the animals. The question of whether or not death was related to the type or amounts of physical activity was evaluated. A rating schedule was designed and the results indicated that no apparent correlation between toxicity and activity was found; however, this does not mean that activity does not affect toxicity. With these mice and these test conditions, the normal pattern of activity produced a slight hyperactivity for the first 15 to 30 min that the animals were placed into the chamber followed by a very inactive condition throughout the remainder of the test whether the animal survived or died. For example, the activity ratings show that an activity rating of two indicated that the animal was very still and perhaps was sleeping. An activity rating of three indicated that the animal was moving about the compartment. The average total physical activity rating per animal for survivors throughout all of the exposure tests was 2.3 and the rating among the animals which died was 2.5. These results indicate that regardless of the type of exposure and whether or not the animals died, they were relatively inactive. In order to test the influence of activity, it will be necessary to produce and regulate physical activity in the animals.

Examinations of the animals used on this program, both gross and histopathological, indicated a similar degree of congestion in the lungs and vascular system of all animals examined, regardless of the level of exposure or the length of survival time. This result is similar to that seen in previous studies. (14) These results are interpreted as an indication that the single and combined exposures produced a disruption of the thermal regulatory center in the brain, probably an anoxic effect resulting in all animals having an elevated temperature and circulatory collapse at the time of death.

The primary purpose of the work completed during this study has been to further define the hazard to human life presented by exposure to combustion products from structural fires. Before this can be done, the results of the animal experiments must be related to humans. In Table 21, we have estimated the equivalent values, in terms of toxicity, for the variables studied. The physiological effects to be expected when humans are exposed to these equivalence levels are outlined in Table 22 for the primary variables only. Such information regarding acute exposures to the noxious gases is not known for humans.

The levels of single and combined variables which caused or are expected to cause death within a 4-hr exposure are summarized in Table 23. The right-hand side of this table indicates the estimated equivalence values for humans.

The results of this study add support to the conclusions found in OCD Work Unit 2537A which indicate the significance of exposures to

TABLE 21. ESTIMATED EQUIVALENCE LEVELS

<u>Variable</u>	<u>Mice</u>	<u>Humans</u>
Oxygen (Anoxia), percent by volume	21 16 13.5 11 7.5	21 17 14 11 8
Temperature, °F	75 85 95 110 120	75 95 110 120 130
Carbon Monoxide, percent by volume	0.050 0.075 0.1 0.150	0.01 0.02 0.03 0.04
Carbon Dioxide, percent by volume	5 10 25 40 50	5 7 10 14 20
Hydrogen Cyanide, percent by volume	10 30 70 100	16 30 70 100
Sulfur Dioxide, percent by volume	25 200 500 800	5 30 70 100
Nitrogen Dioxide, percent by volume	10 30 70 100	10 30 70 100

TABLE 22. HUMAN PHYSIOLOGICAL EFFECTS FROM VARIOUS LEVELS OF ANOXIA, T, CO, CO<sub>2</sub>, AND SELECTED NOXIOUS GASES

Variable	Exposure	Results
Anoxia	8% O <sub>2</sub>	Symptoms become serious and stupor sets in; unconsciousness occurs
	11% O <sub>2</sub>	Nausea and vomiting, exertion impossible, paralysis of motion
	14% O <sub>2</sub>	Dizziness, shortness of breath, headache, numbness, quickened pulse, efforts fatigue quickly
	17% O <sub>2</sub>	Respiration volume increases, muscular coordination diminished, attention and clear thinking require more effort
Temperature	130 °F	Less than 4-hr tolerance time, systemic hyperthermia, peripheral vascular collapse
	120 °F	3- to 5-hr tolerance time
	110 °F	Heat balance cannot be long maintained
	100 °F	Danger of heat prostration and heat stroke
Carbon Monoxide	0.04% CO	Headache after 1 hr, collapse after 2 hr, death after 3 to 4 hr
	0.03% CO	Headache after 1-1/2 hr, collapse after 3 hr
	0.02% CO	Headache after 2 to 3 hr, collapse after 4 to 5 hr
	0.01% CO	No appreciable effect in short exposure, allowable for several hr
Carbon Dioxide	9% CO <sub>2</sub>	Distinct dyspnea, loss of blood pressure, congestion, fatal within 4 hr
	8% CO <sub>2</sub>	Dizziness, stupor, unconsciousness within 4 hr
	7% CO <sub>2</sub>	Marked increase in lung ventilation, headache, dizziness, sweating
	6% CO <sub>2</sub>	Slight physiological effects, noticeable increase in lung ventilation

TABLE 23. EQUIVALENCE LEVELS OF SINGLE AND COMBINED VARIABLES  
CAUSING DEATH IN 4 HR

Variables		Lethal Levels for Mice (for 4-hr exposure)	Estimated Lethal Levels for Humans (for 4-hr exposure)
Single Variable			
T*		120°F	130°F
O <sub>2</sub>		7.5%	8%
CO		0.150%	0.04%
CO <sub>2</sub>		50%	20%
HCN		100 ppm	100 ppm
SO <sub>2</sub>		800 ppm	100 ppm
NO <sub>2</sub>		100 ppm	100 ppm
Combinations			
O <sub>2</sub> + CO + CO <sub>2</sub> + T + HCN		16% O <sub>2</sub> + 0.075% CO + 30% CO <sub>2</sub> + 100°F + 10 ppm HCN	17% O <sub>2</sub> + 0.01% CO + 10% CO <sub>2</sub> + 110°F + 10 ppm HCN
O <sub>2</sub> + CO + CO <sub>2</sub> + T + SO <sub>2</sub>		16% O <sub>2</sub> + 0.075% CO + 30% CO <sub>2</sub> + 100°F + 25 ppm SO <sub>2</sub>	17% O <sub>2</sub> + 0.01% CO + 10% CO <sub>2</sub> + 110°F + 3 ppm SO <sub>2</sub>
O <sub>2</sub> + CO + CO <sub>2</sub> + T + NO <sub>2</sub>		16% O <sub>2</sub> + 0.075% CO + 30% CO <sub>2</sub> + 100°F + 10 ppm NO <sub>2</sub>	17% O <sub>2</sub> + 0.01% CO + 10% CO <sub>2</sub> + 110°F + 10 ppm NO <sub>2</sub>
Combustion Products		20% O <sub>2</sub> + 0.125% CO + 1.3% CO <sub>2</sub> + 100°F + 3 ppm HCN + 0.9 ppm SO <sub>2</sub> + smoke + other trace constituents	20.5% O <sub>2</sub> + 0.03% CO + 0.5% CO <sub>2</sub> + 110°F + 3 ppm HCN + 0.1 ppm SO <sub>2</sub> + smoke + other trace constituents

\*Temperature

combinations of variables likely to be found within the mass fire environment. The variables evaluated in this study were extended to include other noxious gases such as hydrogen chloride, ammonia and phosgene for definition of the components of the combustion atmosphere. In addition, further study is necessary to determine effects of physical activity of the animals in the toxic environment. It is likely that humans in a shelter are not always going to be sleeping or resting. Some are going to be highly active and a definition of their toxicity is essential to an understanding of its importance. The importance of humidity is also considered as a question requiring investigation.

Additional work is also required to show the toxicity of combinations of nitrogen dioxide, sulfur dioxide, and hydrogen cyanide with the primary variables. These were studied in OCD Work Unit 2537B singly, i. e., with one noxious gas and the other four variables. In addition, the noxious gas hydrogen chloride should be included because of its importance in fire atmospheres. It has been suggested by other investigators<sup>(35)</sup> that this gas may be the component responsible for the highly congested lung conditions found following exposure to combustion atmospheres and the deaths which follow such exposure by several days. A chamber designed with a material other than that used in this work unit would be necessary in order to eliminate the hydrogen chloride absorption problem. It is possible that painting of the surface on the inside with a special type of paint would solve this problem.

Much of the preliminary work has been done to define the toxicity of the primary variables singly and in combinations plus some of the noxious gases singly and in combinations. However, exposure tests with the complete combustion product atmospheres are essential to future programs as a means of relating the importance of any single particular identifiable component. It is essential that the components of this atmosphere be accurately measured and monitored during these tests. Equipment needs will be such that various materials can be burned to produce conditions which may simulate the buildup of fire gases and combustion products in a shelter or room. The design of the exposure chamber should be sufficiently large so that static exposures can be conducted. The material should burn to produce a smoky atmosphere within the chamber, the chamber sealed, and the animals inserted for the toxicity evaluation. The equipment used for burning the various materials in this work unit should be designed specifically for the animal experimentation study. As a corollary to the animal experiment, a detailed analysis of the combustion products would be very useful. Analytical methods used during this work unit were limited and not sufficiently accurate nor selective to produce any further definition as is desired. It is likely that gas chromatographic procedures in conjunction with selective detector systems would be useful for monitoring and measuring not only the noxious gases such as hydrogen chloride, sulfur dioxide, hydrogen cyanide, and nitrogen dioxide, but other components which may be of interest in the fire atmosphere. Because of the wide variation of materials likely to be encountered in a fire

situation and the various combinations of burning rate and combinations of different materials, it is obvious that the content of combustion atmospheres can vary widely. A detailed analysis is essential for determinations of the type of gases likely to be encountered.

Further study to determine the effect that increased physical activity may have on the relative toxicity to the various components of smoke could be accomplished by several procedures. One possibility is to introduce a squirrel-type cage which turns, inducing the mice to run at various speeds. In this way, activity of various stages could be introduced as a controlled variable.

In conjunction with each of the animal tests, the same animal strain and procedures as used on previous programs for histopathological examination should be followed, including the gross examination followed by blocking of the tissues from vital organs and preparation of slides for histopathological study.

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Andrew J. Pryor, Donald E. Johnson, Nathan N. Jackson

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In previous studies, the significance of synergistic action brought about by animal exposures to combinations of elements found within the combustion products in a mass fire environment was identified. An experimental program was undertaken to further define the life hazard in a mass fire environment resulting from exposures to these combustion products. Studies included exposures to combinations including the variables of carbon monoxide, temperature, oxygen, carbon dioxide, sulfur dioxide, nitrogen dioxide, hydrogen cyanide, the presence of smoke (particulate matter), and all of the trace constituents to be found. The data from these tests and the results of the histopathological studies were reviewed in an effort to define the significance of human exposure to combustion products as found within the mass fire environment.